N64 81857

EVALUATION OF LIQUID STERILANTS PHASES III, IV, V, VI, AND VII. JOHN B. OPFELL, ET AL. JET PROPULSION LAB., PASADENA, CALIF. NAS7-100. 110P. 7 SEP 62.

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21 DOCUMENT CLASS: SINGLE [X] CONTENTS NOTE [ ] ANALYTIC [ ] NUM EACH [ ] NUM SEL [ ] NUM PRIMARY [ ] YES [ ] NO [ ] ANALYTIC SUBSIDIARY [ ] PRIMARY ACCESSION NUMBER [ ] SELECTED ITEM [ ]

22 EVAL INITIALS: 5/1964

23 ABSTRACT CLASS: ENGLISH [ ] AUTH [X] FAC [ ] FOR [ ] LANGUAGE [ ] END [ ] FOR. [ ] FAC. [ ]

ACCESSING

24 NO OF PAGES: 109

25 ODD SIZE INCLUSION: YES [ ] NO [X]

26 DOCUMENT: INCOMP [ ] NOT REPRO [ ]

27 SEND TO OTS: YES [ ] NO [X]

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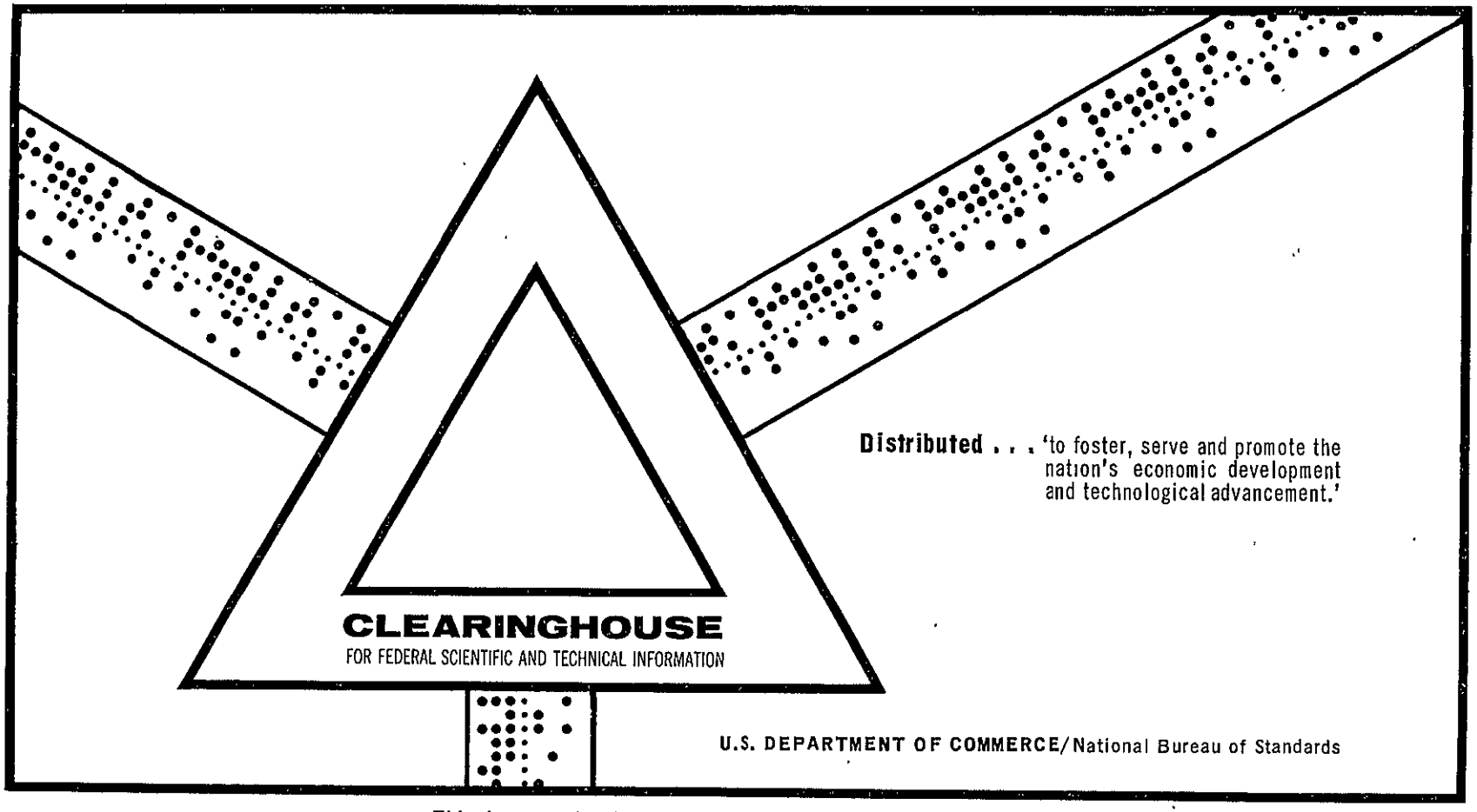
EVALUATION OF LIQUID STERILANTS - PHASES III, IV, V, VI, AND VII

John B. Opfell, et al.

Dynamic Science Corporation
South Pasadena, California

7 September 1962



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FINAL REPORT

EVALUATION OF LIQUID STERILANTS

PHASES III, IV, V, VI, AND VII

Submitted in fulfillment
of
Jet Propulsion Laboratory
Contract N2-150247

Prepared by:

John B. Opfell, Ph.D.
Curtis E. Miller, M.D.
Allan L. Louderback, Ph.D.
Edward G. English
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Mr. Rolf C. Hastrup
Senior Engineer
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, California

Dear Mr. Hastrup:

Enclosed are 25 copies and a reproducible master of our Final Report on "Evaluation of Liquid Sterilants, Phases III, IV, V, VI, and VII" submitted in fulfillment of Jet Propulsion Laboratory Contract No. N2-150 247 with Dynamic Science Corporation.

This Final Report supplements our Semifinal Report (March 16, 1962) and discusses the following general topics:

(1) Stability of the formaldehyde-in-absolute-methanol liquid sterilant;

(2) A liquid sterilant based on ethylene oxide;

(3) Stability of the grease sterilant;

(4) Long term compatibility of liquid and grease sterilants with electrical components;

(5) Identification of factors having a critical effect on efficacy of liquid sterilants; and

(6) Corrections of errors in earlier reports.

Mr. Rolf C. Hastrup

The information supplied on the efficacy of ethylene oxide in methanol as a liquid sterilant was developed in work supported by Hughes Aircraft Company under Contract No. 4-681981-FF 36-6 with Dynamic Science Corporation. This information is presented with the permission of Hughes Aircraft Company.

Very truly yours,

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C. E. Miller, M. D.

C. E. Miller, M. D.
Director of Medical Sciences

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FINAL REPORT

EVALUATION OF LIQUID STERILANTS

PHASES III, IV, V, VI, AND VII

(Submitted in fulfillment of Jet Propulsion Laboratory Contract N2-150247)

Prepared for: Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, California

SEPTEMBER 7, 1962

Approved by: Melvin Gerstein
Melvin Gerstein, Ph.D.
Vice President

M. Edmund Ellion, Ph.D.
President

This work was performed for the Jet Propulsion Laboratory, California Institute of Technology, sponsored by the National Aeronautics and Space Administration under Contract NAS7-100.

DYNAMIC SCIENCE CORPORATION
1445 Huntington Drive
South Pasadena, California

Report No. R-5 of P-44B

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

I. INTRODUCTION

The information presented here supplements that presented in the Semifinal Report. At the time of preparation of the Semifinal Report, the following questions were still unanswered because of the time required to complete long term surveillance tests.

(1) Is a stable, sterilizing, and noncorrosive electrical grease feasible?

(2) Are liquid sterilants harmful to the performance of electrical connectors and electrical insulating materials when in contact with them for long periods of time?

Portions of the measurements in Phase V have been repeated and the results are reported. The general conclusions drawn earlier have been confirmed in these additional measurements.

Some exploratory measurements of the effectiveness of a liquid sterilant based on ethylene oxide are reported. The need for scrupulous cleanliness if sterility is to be achieved through the use of liquid, gas, or grease sterilants is to be emphasized.

II. PHASE III

A. STABLE LIQUID FORMALDEHYDE STERILANT

1. The Sterilization Effectiveness of Formaldehyde in Absolute Methanol After Storage

A factorial experiment was designed and performed to determine the relationship of the following factors to the effectiveness of the formaldehyde-in-absolute-methanol liquid sterilant.

a. Storage temperatures (2-4°C, 20-22°C, 37°C)

b. Storage containers, (clear glass, brown glass and polyethelene)

c. Exposure times, (1 hour, 3 hours)

d. Spore concentrations (10^6, 10^7, 10^8 cells)

For purpose of abbreviated description, the 5% $^{w}/_{v}$ formaldehyde-in-absolute-methanol prepared in the manner discussed in the Semifinal Report [1] will be called Liquid Sterilant MA5F.

Glass slides which had been sterilized by baking in Petri dishes for 3 hours at 165°C, were inoculated with 10^6, 10^7, and 10^8 spores of *B. subtilis, var. niger* which were suspended in 0.01 ml of distilled water. After drying, the inoculums were exposed to the sterilant. Using a syringe, 0.40 ml of the sterilant were applied over the inoculum. Inoculums were subjected to the sterilization treatment for both 1 and 3 hours. After the exposure period ended, the slides were each transferred in a sterile manner, to 50 ml of sterile distilled water. Each slide was scrubbed in the water by sonication for one minute to remove the spores from the surface of the glass slide and suspend them uniformly in the water. After scrubbing,

a 0.2-ml aliquot of the water was removed aseptically with a sterile syringe and placed on a Trypticase soy agar plate/Hyland. All plates so prepared were incubated for 7 days at 37 $\pm$ 2°C and the colonies which developed were then counted.

Table I summarizes the results of the assay done shortly after the sterilants were first prepared. Table II presents the results of a similar assay performed after the sterilants had aged for 1 month in the several containers and at the several temperatures. The data presented in Table I show that at 3 hours the sterilant exhibits sufficient sterilizing effectiveness.

The sterilizing-effectiveness stability of the sterilants in each of the several containers and temperatures was evaluated by comparison of the initial assays with those made after 1 month. The data in Table I show all of the different specimens of the sterilant to be effective in a 3-hour exposure while Table II shows that none of the 3-hour exposure periods with the aged sterilant produced sterility. Walker[2] indicates that even in a nonaqueous-methanol environment formaldehyde can polymerize to form paraformaldehyde but that the rate of polymer formation is very slow compared to the rate of polymerization of formaldehyde in water. This polymerization may be contributing to the reduction in sterilizing effectiveness.

A general pattern appears in Table II to the extent that the sterilants stored at 37°C appear to have been more effective than were the sterilants at 4°C and 21°C. To explore this pattern additional exposures were made at 21°C and 37°C. Table III summarizes the data from this experiment.

In contrast to the situation represented in Table II, the sterilant was completely effective at 37°C. The same degree of effectiveness at 21°C is shown in Table III as was shown in Table II.

Because formaldehyde polymer is less stable at higher temperatures, even in aqueous solutions, an attempt was made to reverse the paraformaldehyde formation by refluxing [2] the sterilant for various periods of time. Three-milliliter samples of the sterilant stored in the brown bottle in the refrigerator were sealed in glass tubes and were refluxed for 15, 30, and 60 minutes respectively. The sterilization effectiveness of the refluxed sterilants were then assayed along with those of the same sterilants which had not undergone any treatment. Also, the sterilants which had not been refluxed were compared in their effect on spore viability when the exposure was at both 37°C and 21°C. Table IV summarizes the results of this experiment. The time of reflux not only failed to increase effectiveness at room temperature, but quite the opposite, the sterilants became less effective with longer refluxing. The reason for this is not apparent. After a 3-hour exposure the sterilant at 37°C caused complete sterilization of all levels of inoculum studied as had been the case in the experiments reported in Table III.

The experiments described above indicate that when the Liquid Sterilant MA5F is freshly prepared, it is effective in a 3-hour exposure at 21°C (room temperature), but that after storage for 1 month, it is much less effective under these same conditions. A longer period at room temperature may be effective. In a 3-hour exposure at 37°C the sterilant is still quite effective.

When fresh Liquid Sterilant MA5F was prepared at a later date, part of the preceeding experiment was repeated. Glass slides were sterilized and inoculated as described earlier. The sterilant was applied in the same manner, except that 0.50 ml of sterilant was used. The remaining procedure followed was that described earlier except that a 0.4-ml aliquot of the rinse water was taken aseptically and placed on each agar plate. A recovery control was prepared in which a slide, inoculated with 10^6 spores, was left unexposed to sterilant, but was sonicated in water and a 0.4-ml aliquot of the rinse placed on the nutrient agar. The assay results are presented in Table 1b and confirm the earlier experience with the effectiveness of freshly prepared Liquid Sterilant MA5F.

The contact time between Liquid Sterilant MA5F and the contamination will generally be on the order of weeks in practical application. During this time some polymerization will occur. The exposure of the microorganisms will be long enough such that the effectiveness of Liquid Sterilant MA5F will be similar to that of Grease Sterilant S4C5PF. Even though month-old Liquid Sterilant MA5F contains polymers, it evaporates complete in air in a few hours. This property is useful in case of small spills onto other spececraft components. The anhydrous formulation of the sterilant in methanol will minimize corrosion[3] resulting from autooxidation of the formaldehyde to formic acid.

B. A LIQUID STERILANT OF ETHYLENE OXIDE IN ABSOLUTE METHANOL

1. The Chemistry of Ethylene Oxide in Methanol

Ethylene oxide may polymerize[4] in the presence of anhydrous iron, tin, and aluminum chlorides; metallic potassium; pure iron; aluminum oxides; alkali metal hydroxide; acids; and organic bases. In certain instances the polymerization is immediate; in others, it is very slow. The rate of polymerization[5] is increased by elevated temperature and by high surface-to-volume ratio of the containing vessel or system. Typically, zinc, cadimum, chromium, and tin electroplates and acid-pickled steel promote rapid polymerization.

In the presence of appropriate catalysts ethylene oxide will react with water to form ethylene glycol and with methanol to form methoxyethanol, the monomethyl ether of ethylene glycol. Characteristically, ethylene oxide reaction products are nonionic so that they do not contribute to corrosion and changes in electrical properties to a significant degree. The compatibility of ethylene oxide with many materials is a result of this property and the small amount of ethylene oxide available for reaction.

2. Preparation of Ethylene Oxide-in-Methanol Sterilant

Because the boiling point of a liquid mixture depends upon its composition, ethylene oxide can be made conveniently available as a liquid sterilant by dispersing in it absolute methanol. The boiling point of 5% v/v ethylene oxide in absolute methanol is above room temperature although the exact value is not known. A 5% v/v ethylene oxide in water solution boils at 38°C and a 10% solution boils at 23°C.[6]

While ethylene oxide normally boils at 10.73°C it can be handled, in a hood, conveniently as a liquid. (Because of the fire, toxicity, and explosion hazards associated with the vapors, only small amounts are handled at any one time). Ethylene oxide can be measured in glass graduates and volumetric flasks without any particular care given to temperature control. Cooling glassware introduces a serious moisture condensation problem.

A 5% v/v ethylene-oxide-in-absolute-methanol solution can be prepared conveniently by standard volumetric methods. It is useful to introduce the liquid ethylene oxide into the absolute methanol when transferring it from the volumetric measuring vessel. The 5% v/v concentration was studied for effectiveness in order to use a formulation comparable to that of the Liquid Sterilant MA5F. Concentrations of 10% v/v and even 20% v/v may be substantially more effective and sufficiently compatible with many materials. The effect of the state of hydration of spores [7] on the sterilizing effectiveness of ethylene oxide in absolute methanol has not been explored.

The nonvolatile residue-of-polymers content of the ethylene oxide used to prepare the sterilant must be measured and controlled. A gravimetric procedure has been found to be sufficient.

3. Effectiveness of Ethylene-Oxide-in-Methanol Sterilant

The possibility of using ethylene oxide in methanol as a sterilizing agent was explored. A 5% v/v solution of ethylene oxide in absolute methanol was prepared and tested in parallel with the Liquid Sterilant MA5F discussed in the preceding section. The experimental conditions described in Section A were used in testing the effectiveness of the ethylene-oxide-in-methanol liquid

sterilant. The inoculums of spores were deposited on previously sterilized glass slides in Petri dishes. The length of exposure, the scrubbing method, and the dilution factors were the same as in the earlier measurement. Table V summarizes the observations of sterilization effectiveness of ethylene oxide in absolute methanol. These observations indicate that the sterilant is not effective in an exposure as short as 3 hours.

One month after the ethylene oxide in absolute methanol sterilant had been prepared its effectiveness in a 24-hour exposure at room temperature was measured. The sterilant-treated spores on glass slides were placed inside of polyethylene bags in order to maintain the evaporated ethylene oxide in contact with the spores. Table VI presents the results of this experiment. The ethylene-oxide liquid sterilant is partially effective under these exposure conditions. The 24-hour exposure is more realistic for ethylene oxide than was the 3-hour exposure used in the development of the data in Table V. However, the effectiveness of ethylene oxide in absolute methanol as a liquid sterilant is still less than that of Liquid Sterilant MA5F. Because of its compatibility with cetain components, ethylene oxide in absolute methanol may, however, have a place in the sterilization of spacecraft if the temperature and concentrations are sufficiently high and the exposure sufficiently long.

4. Assay of Ethylene Oxide in Methanol

A chromatographic assay of ethylene oxide has been described by Johns[8]. The method used here to verify composition has been an extension of that used for process control on gas sterilization with ethylene oxide. The chromatographic column is packed with 20% silicone grease on firebrick and the column is operated at 150°C. Bombaugh and Bull[9] have described a gas chromatographic assay procedure for formaldehyde in water and/or methanol.

C. A STABLE GREASE STERILANT

1. The Sterilizing Effectiveness of Paraformaldehyde in Dow-Corning #4 Compound After Storage

For purposes of convenience, the grease sterilant compound of 4.76% w/w paraformaldehyde in Dow-Corning #4 Compound and prepared in accordance with the procedures described in the Semifinal Report[2] will be called Grease Sterilant S4C5PF. The original batch of this material was stored in sealed jars at room temperature until 6 months after its preparation. The effectiveness of this sterilant was measured in precisely the same manner as its effectiveness had been measured when it was first prepared.

The three stock B. subtilis, var. niger spore suspensions were confirmed, by assay, to contain 10^{10}, 10^{9}, and 10^{8} viable spores per ml assayed. Each of the 0.01-ml inoculums of each of these suspensions were placed on a sterile bead at the end of a glass rod and allowed to dry. After drying, the inoculums on the beads were covered with the Grease Sterilant S4C5PF. Then the sterilant-treated beads were plucked from the rods and placed aseptically into sterile baby food jars and left there for 48 hours. When this exposure period was terminated, the spores were scrubbed, by sonication, from the glass beads and grease with 20 ml of reagent grade trichloroethylene which had been sterilized by passing it through a sterile Millipore filter. One ml of the rinse solvent was transferred aseptically into 9 ml of sterile water. From this mixture 0.2 ml of the aqueous phase were taken to inoculate Trypticase soy agar/Hyland plates. The plates were then incubated at 37°C for 4 days, at the end of which time the colonies present were counted and are reported in Table VII.

After the extended storage period, the Grease Sterilant S4C5PF showed no signs of physical deterioration.

A second batch of Grease Sterilant S4C5PF was prepared and assayed for effectiveness by the same procedures. The results of this assay are also reported in Table VII. This second batch of material had not been exposed to 150°C for 1 hour as the first batch had been.

The grease sterilant appears to be both highly effective and stable.

2. Effectiveness In Applications Involving Partial Wetting

The Grease Sterilant S4C5PF has one drawback, which may be a consequence of its stability. It is not effective in partial wetting experiments[1] though it is effective in killing secondary contaminants. It may be that its effectiveness in partial wetting applications can be increased substantially by exposure to temperature on the order of 35°C for extended periods of time. This possibility has not been explored.

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8. Johns, T. "Analysis of Ethylene Oxide Sterilizing Mixtures by Gas Chromatography," Application Data Sheet GC-98F Fullerton, California: Beckman Instruments, Inc. (1959).

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PHASE III

TABLE I

Effectiveness of Freshly-Prepared Liquid Sterilant MA5F in Killing Spores of B. subtilis, var.niger

Colonies of B.subtilis, var.niger*

Bottle type	Storage Temp. degrees C.	10^6 spores+		10^7 spores+		10^8 spores+	
		1 hr. exposure	3 hr. exposure	1 hr. exposure	3 hr. exposure	1 hr. exposure	3 hr. exposure
Brown glass	4	256	0	TNC	0	TNC	0
	21	1	0	TNC	0	TNC	0
	37	248	37	TNC	1	TNC	22
Clear glass	4	248	1	TNC	2	TNC	2
	21	TNC	0	TNC	0	TNC	0
	37	TNC	0	TNC	0	TNC	0
Polyethylene	4	25	0	620	0	TNC	0
	21	125	0	TNC	0	TNC	0
	37	356	0	TNC	0	TNC	0

* After 7 days at 37± 2°C on Trypticase soy agar/Hyland.

\+ If all spores developed colonies when incubated on Trypticase soy agar/Hyland, the expected number of colonies developing would be 0.004 times the indicated inoculum size.

PHASE III

TABLE Ib

Effectiveness of Freshly-Prepared Liquid Sterilant MA5F in Killing Spores of B. subtilis, var. niger*

Treatment	Spore Inoculum+	Colonies of B. subtilis, var. niger
1 hour exposure	10^6	712
	10^7	TNC
	10^8	265
3 hour exposure	10^6	0
	10^7	0
	10^8	0
control	10^6	TNC

* After 7 days at $37 \pm 2^{\circ}C$ on Trypticase soy agar/Hyland.

\+ If all spores developed colonies when incubated on Trypticase soy agar/Hyland, the expected number of colonies developing would be 0.008 times the indicated inoculum size.

PHASE III

TABLE II

Effectiveness of One-Month Old Liquid Sterilant MA5F in Killing Spores of B. subtilis, var. niger

Colonies of B. subtilis, var. niger*

Bottle type	Storage Temp. degrees C.	10^6 spores+		10^7 spores+		10^8 spores+	
		1 hr. exposure	3 hr. exposure	1 hr. exposure	3 hr. exposure	1 hr. exposure	3 hr. exposure
Brown glass	4	TNC	2	TNC	484	TNC	TNC
	21	30	TNC	TNC	TNC	TNC	TNC
	37	0	1	620	TNC	TNC	560
Clear glass	4	203	528	772	884	TNC	254
	21	2	130	TNC	332	130	372
	37	424	17	17	184	TNC	378
Polyethylene	4	288	250	350	282	TNC	TNC
	21	149	157	97	TNC	TNC	394
	37	205	59	856	302	31	216

* After 7 days at 37$\pm$ 2°C on Trypticase soy agar/Hyland.

\+ If all spores developed colonies when incubated on Trypticase soy agar/Hyland, the expected number of colonies would be 0.004 times the indicated inoculum size.

PHASE III
TABLE III

Effectiveness of Liquid Sterilant MA5F, after One-Month of Storage at Room Temperature, in Killing Spores of B. subtilis, var. niger

(Three-hour Exposure to Sterilant)

Colonies of B. subtilis, var. niger *

Bottle type	Temp. of Inoculum during exposure	10^6 spores+	10^7 spores+	10^8 spores+
Brown glass	21°C	49	TNC	TNC
	37°C	0	0	0
Clear glass	21°C	449	606	TNC
	37°C	0	0	0
Polyethlene	21°C	2	39	TNC
	37°C	0	0	0

* After 7 days at 37 ± 2°C on Trypticase soy agar/Hyland

+ If all spores developed colonies when incubated on Trypticase soy agar/Hyland, the expected number of colonies would be 0.004 times the indicated inoculum size.

PHASE III

TABLE IV

Sterilization Effectiveness of Liquid Sterilant MA5F, Stored at 4°C in a Brown Bottle on Spores of B. subtilis, var. niger

(Three-hour Exposure to Sterilant)

Colonies of B. subtilis, var. niger*

Time Sterilant was refluxed, Minutes	Temperature of Exposure, °C	Inoculum+		
		10^6	10^7	10^8
0	21	104	300	76
0	37	0	0	0
15	21	109	TNC	TNC
30	21	TNC	TNC	TNC
60	21	TNC	TNC	TNC

* After 7 days at 37±2°C on Trypticase soy agar/Hyland.

\+ If all spores developed colonies when incubated on Trypticase soy agar/Hyland the expected number of colonies developing would be 0.004 times the indicated inoculum size.

PHASE III

TABLE V

Sterilization Effectiveness of 5% v/v Ethylene Oxide in Absolute Methanol on Spores of B. subtilis, var. niger

(Freshly Prepared Solutions Were Used)

Colonies of B. subtilis, var. niger*

Bottle type	Storage Temp., degrees C.	Inoculum+					
		10^6 spores		10^7 spores		10^8 spores	
		1 hr. exposure	3 hr. exposure	1 hr. exposure	3 hr. exposure	1 hr. exposure	3 hr. exposure
Brown glass	21	640	368	TNC	TNC	TNC	TNC
Clear glass	21	308	TNC	TNC	196	640	TNC
Polyethylene	21	312	248	TNC	TNC	TNC	TNC

* After 7 days at $37\pm2^{\circ}C$ on Trypticase soy agar/Hyland.

\+ If all spores developed colonies when incubated on Trypticase soy agar/Hyland the expected number of colonies developing would be 0.004 times the indicated inoculum size.

PHASE III

TABLE VI

Sterilization Effectiveness of 5% v/v Ethylene Oxide in Absolute Methanol on Spores of B. subtilis, var. niger

(Measurements were made after solution was stored for one month, the exposure was for 24 hours)

Colonies of B. subtilis, var. niger*

Bottle type	Storage Temp., degrees C.	Inoculum+					
		10^6 spores		10^7 spores		10^8 spores	
Brown glass	21	1	75	179	337	340	634
Clean glass	21	19	2	80	1	TNC	245
Polyethylene	21	137	1	TNC	71	TNC	420

* This group was kept inside of two plastic bags to keep the ethylene oxide from escaping from the immediate environment.

\+ If all spores developed colonies when incubated on Trypticase soy agar/Hyland the expected number of colonies developing would be 0.004 times the indicated inoculum size.

PHASE III

TABLE VII

Effect of Storage on Effectiveness of Mixtures of Paraformaldehyde in Dow-Corning #4 Compound in Sterilizing B. subtilis, var. niger* spores

Concentration of Paraformaldehyde	Storage Time	Specimen Inoculum						Bacteriostasis Inoculum					
		10^6		10^7		10^8		10^4					
		a^+	b^+	a^+	b^{++}	a^+	b^+	a_1^+	a_2^+	a_3^+	b_1^+	b_2^+	b_3^+
0%	fresh	15	77	800	TNC	TNC	TNC	3			6		
	6 months**	13		133		TNC							
4.76%	fresh	0	0	1^{++}	0	2	23	8			6		
	6 months	0	0	0	0°	1	0	0.	0	397	1	0	0
4.76%§	fresh	0	0	0	0	0	0	0					

* After four days at $37 \pm 2^{\circ}C$ on Trypticase soy agar/Hyland.

+ If all spores developed when incubated on Trypticase soy agar/Hyland, the expected number of colonies developing would be 0.001 times the indicated inoculum size. a and b are replicates.

** In this case, instead of 0% Paraformaldehyde in the grease, no grease at all was applied to the slides.

++ Possibly contaminant.

§ This grease sterilant represents a second batch which was not treated at $150^{\circ}F$ for one hour during its preparation.

III. PHASE IV

A. CONTACT RESISTANCES OF PHASE IV SPECIMENS

The contact resistances for subjects r, s, and t were measured 27 weeks (6 months) after exposure to liquid sterilants E, F, G, and H, where:

E is 5% v/v Ethylene imine in absolute methanol.

F is 5% w/v Formaldehyde in absolute methanol.

G is 5% v/v Beta-propiolactone in absolute methanol.

H is 4.76% w/w Paraformaldehyde in Dow-Corning #4 compound.

Immediately after the insulation resistance measurements described in the Semifinal Report[1] had been completed on each of these subjects, the sterilant was reapplied, the connector parts were mated and then stored in polyethylene bags at room temperature.

The results of the measurements are reported in Table VII-f and the analysis of variance in the measurements is reported in Table VIII-f. During storage the specimens of subjects r, s, and t were wrapped individually in 1-mil thick polyethylene bags to prevent cross contamination with sterilants. The connectors were mated during the entire 27 weeks storage period. They were stored at room temperature (20-24°C).

After 27 weeks of storage the contact resistances of the Phase IV specimens were measured in a manner identical to that described in Section A, Phase IV of Semifinal Report[1].

At the 1% level of significance both the treatments (before and after storage) and the sterilants are significant. The T_* values for the sterilants indicate that Sterilant E caused very large changes to the contact resistance during the storage period. Comparison with the earlier Analysis of Variance, Phase IV, Table VII, Semifinal Report[1], indicates that continued exposure to Sterilants F and H did not affect the contact resistance during this storage period. Since the results of this surveillance study shows that Sterilants E and G cause large changes in contact resistance their use cannot be recommended for sterilizing these subjects.

B. INSULATION RESISTANCES OF PHASE IV SPECIMENS

The electrical resistances of the insulations in subjects r, s, and t were measured 27 weeks (6 months) after exposure to liquid sterilants E, F, G, and H. Table IX-f presents the galvanometer deflection readings and shunt settings which are related to the insulation resistances in the manner described earlier[1,2] These readings were converted to resistance, in ohms, and in Table X-f are presented as $\log_{10}$ resistances.

During storage in contact with the liquid sterilants the specimens were mated and stored individually in 1-mil thick polyethylene bags. The storage temperature was 20-24°C (room temperature).

The electrical resistances of the insulation in subjects r, s, and t were measured in a manner identical to that described in Section V, Phase IV, Semifinal Report[1].

The analysis of variance, Table XI-f, shows, as it did with the 24-hour measurements that at the 1% level of significance there is a greater disparity in effects among the subjects than among the sterilants or the treatments although all are significant. A comparison of this analysis of variance with that in Table XI of Phase IV, Semifinal Report[1] shows that the greatest effects of the sterilants on insulation resistances took place within the first 24 hours. During the continued contact with the sterilants, the deviations from the original before treatment values partially disappeared.

The measurement technique could not discriminate among resistances greater than 10^{13} ohms. The maximum loss in resistance observed, still left a resistance of more than 10^{7} ohms. The acceptability of resistances of this magnitude was not determined within the scope of this study.

C. INSULATION RESISTANCE OF SEVERAL INSULATED WIRES AND A POTTING COMPOUND.

Using exactly the same technique as that described in the Semifinal Report[1], the insulation resistances of subjects u through z' were measured again 27 weeks after the initial exposure to the liquid sterilants. The specimens had been treated with the sterilants E, F, G, and H immediately after the "after-treatment" measurements, reported in the Semifinal Report were completed. The specimens were stored individually in 1-mil thick polyethylene bags at room temperature. Table XIII-f and XIV-f report the results of measurements of the insulation resistances.

The insulation resistances of these specimens were measured in a manner identical with that reported in Section F, Phase IV, Semifinal Report[1]. A comparison on the information in Tables XIII-f and XIV-f with that in Tables XIII and XIV, of the Semifinal Report, shows no significant changes in insulation resistance of the specimens to have occurred during storage for 27 weeks in contact with the sterilants.

D. APPLICABILITY OF INFORMATION ON COMPATIBILITY OF STERILANTS WITH MATERIALS IN EVALUATING ELECTRICAL CONNECTORS FOR STERILE SPACECRAFT APPLICATIONS.

Table XVI-f describes the appearances of subjects r through z' 27 weeks after treatment with Sterilant E. Table XVII-f summarized the information gained in the "Evaluation of Liquid Sterilants" studies pertaining to the compatibility of several liquid sterilants with a variety of materials. Table XVIII-f presents a list of materials commonly used in electrical connectors, one type of item which may be sterilized by the liquid-sterilant technique. Many of these materials have not been evaluated compatibilitywise.

In several instances, the electrical properties of the connectors changed by huge amounts during the 27-weeks storage period, indicating that surveillance tests for "qualified components" evaluations should be longer than several days exposure to the sterilants.

The formaldehyde-in-absolute-methanol sterilant continues to look satisfactor for many applications. Of course, "qualified components" evaluations must still be performed on a statistically significant number of each component of interest

after the component has been exposed to a specified sterilant (including age) under specified conditions before the components can be expected to perform reliably in their eventual application.

E. REFERENCES, PHASE IV

1. Opfell, J.B., C.E. Miller, and P.N. Hammons. "Evaluation of Liquid Sterilants, Phases I and II, "Final Report on Jet Propulsion Laboratory Contract No. N1-143452, South Pasadena, California: Dynamic Science Corporation, (August 26, 1962).

2. Opfell, J.B., C.E. Miller, and A.L. Louderback. "Evaluation of Liquid Sterilants, Phases III, IV, V, VI, and VIII," Semifinal Report on Jet Propulsion Laboratory Contract No. N2-150247, South Pasadena, California: Dynamic Science Corporation (March 16, 1962).

PHASE IV

TABLE VII - f

Contact Resistance, 27 Weeks After Treatment

STERILANT E - 5% v/v Ethylene imine in Methanol

Subject	Replicate	Pin no.	Ohms	%RH	Temp, °F	Change, Percent
r	1	2	0.00249	62	68	7.3
		3	0.00224			7.2
		4	0.00301			32.0
		5	0.00230			5.5
		6	0.00235			6.3
	2	2	0.00238	62	68	-0.8
		3	0.00249			10.2
		4	0.00226			0.9
		5	0.00245			4.3
		6	0.00271			12.0
s	1	2	0.0363	62	68	1406.2
		3	0.0262			948.0
		4	0.00502			51.2
		5	0.00515			95.1
		6	0.161			7251.6
	2	2	0.00442	62	68	78.2
		3	0.00216			-17.2
		4	0.00205			-20.5
		5	0.00588			128.8
		6	0.00495			74.3
t	1	2	0.153	63	69	8743.9
		3	0.142			8155.8
		4	0.0715			4454.1
		5	0.0225			1288.9
		6	0.00937			454.4
	2	2	0.0302	63	69	1440.8
		3	0.0556			2905.4
		4	0.0138			721.4
		5	0.00464			144.2
		6	0.0338			1820.5

TABLE VII - f (Continued)

Contact Resistance, 27 Weeks After Treatment

STERILANT F - 5% w/v Formaldehyde in Methanol

Subject	Replicate	Pin no.	Ohms	%RH	Temp, °F	Change Percent
r	1	2	0.00242	62	68	4.8
		3	0.00228			1.3
		4	0.00240			3.9
		5	0.00248			3.8
		6	0.00231			2.7
	2	2	0.00256	62	68	-1.9
		3	0.00251			4.6
		4	0.00285			18.8
		5	0.00259			5.3
		6	0.00249			0.0
s	1	2	0.00244	62	68	10.9
		3	0.00357			39.5
		4	0.00310			19.2
		5	0.00293			-1.7
		6	0.00248			-26.6
	2	2	0.00245	62	68	14.0
		3	0.00287			19.6
		4	0.00245			4.7
		5	0.00258			2.4
		6	0.00262			-16.8
t	1	2	0.00212	62	68	40.4
		3	0.00212			23.3
		4	0.00222			33.7
		5	0.00233			62.9
		6	0.00224			14.3
	2	2	0.00433	62	68	110.2
		3	0.00207			2.5
		4	0.00218			18.5
		5	0.00587			143.5
		6	0.00869			352.6

PHASE IV

TABLE VII - f (Continued)

Contact Resistance, 27 Weeks After Treatment

STERILANT G - 5% v/v Beta-propiolactone in Methanol

Subject	Replicate	Pin no.	Ohms	%RH	Temp, °F	Change Percent
r	1	2	0.00228	63	69	1.8
		3	0.00246			7.9
		4	0.00225			0.0
		5	0.00243			1.3
		6	0.00244			0.0
	2	2	0.00352	63	69	-9.0
		3	0.00225			-0.4
		4	0.00214			-4.9
		5	0.00220			0.5
		6	0.00227			-0.4
s	1	2	0.00477	63	69	194.4
		3	0.0485			2671.4
		4	0.0180			1317.3
		5	0.00213			54.3
		6	0.0187			-7.0
	2	2	0.00327	63	69	45.3
		3	0.0431			3069.1
		4	0.00210			14.1
		5	0.00483			112.8
		6	0.00212			-19.1
t	1	2	0.00578	63	69	197.9
		3	0.00262			44.0
		4	0.0142			603.0
		5	0.0232			1368.4
		6	0.00500			189.0
	2	2	0.00397	63	69	99.5
		3	0.0143			736.3
		4	0.00302			73.6
		5	0.00657			245.8
		6	0.0126			573.8

PHASE IV

TABLE VII - f (Continued)

Contact Resistance, 27 Weeks After Treatment

STERILANT H - 4.76% w/w Paraformaldehyde in Dow-Corning #4 Compound

Subject	Replicate	Pin no.	Ohms	%RH	Temp, °F	Change Percent
r	1	2	0.00230	63	69	-5.0
		3	0.00218			-2.7
		4	0.00225			-1.7
		5	0.00239			4.8
		6	0.00217			-2.7
	2	2	0.00207	63	69	0.0
		3	0.00243			-1.2
		4	0.00238			2.6
		5	0.00225			-5.5
		6	0.00233			0.4
s	1	2	0.00291	63	69	85.4
		3	0.00224			67.2
		4	0.00263			1.9
		5	0.00232			-51.9
		6	0.00214			-6.6
	2	2	0.00242	63	69	-11.7
		3	0.00220			-10.0
		4	0.00239			-0.8
		5	0.00227			-20.4
		6	0.00250			8.7
t	1	2	0.00194	63	69	5.4
		3	0.00188			15.3
		4	0.00205			4.6
		5	0.00167			2.5
		6	0.00190			73.4
	2	2	0.00183	63	69	2.8
		3	0.00170			-1.8
		4	0.00216			25.6
		5	0.00193			-0.5
		6	0.00181			-3.2

TABLE VII - f (Concluded)

Contact Resistance, 27 Weeks After Treatment

CONTROLS

Subject	Replicate	Pin no.	Ohms	%RH	Temp, °F	Change Percent *
r	1	2	0.00245	52	71	5.6
		3	0.00306			46.4
		4	0.00252			10.5
		5	0.00267			22.5
		6	0.00247			11.8
	2	2	0.00237	52	71	-1.3
		3	0.00239			5.8
		4	0.00257			14.7
		5	0.00245			4.3
		6	0.00270			11.6
s	1	2	0.00245	59	70	1.7
		3	0.00344			37.6
		4	0.00277			-16.6
		5	0.00213			-19.3
		6	0.00221			0.9
	2	2	0.00287	59	70	15.7
		3	0.00263			0.8
		4	0.00268			3.9
		5	0.00277			7.8
		6	0.00324			14.1
t	1	2	0.00180	64	71	4.0
		3	0.00201			16.9
		4	0.00182			15.9
		5	0.00198			22.2
		6	0.00184			8.9
	2	2	0.00181	64	71	-7.7
		3	0.00169			-8.6
		4	0.00189			12.5
		5	0.00158			-16.8
		6	0.00214			21.6

* Calculated using the "before treatment" controls of Sterilant E.

PHASE IV

TABLE VIII-f

Analysis of Variance of Contact Resistances, Before and 27 Weeks After Treatment Subjects r, s, and t

FACTOR	LEVEL	n_*	T_*	$\sum T_*^2/n_*$	S_*	df	MS
Subject	r	80	19065	1290	2022	2	1011
	s	80	54827				
	t	80	75248				
Replicate	1	120	99720	10322	1054	1	1054
	2	120	49420				
Pin No.	2	48	33464	9915	647	4	162
	3	48	42135				
	4	48	21988				
	5	48	17055				
	6	48	34498				
Treatment	Before	120	26197	13168	3900	1	3900
	After	120	22943				
Sterilant	E	60	88059	15364	6096	3	2032
	F	60	15482				
	G	60	32415				
	H	60	13184				
Residuals	–	–	–	–	67081	228	294
Total		240	149140	9268	80810	239	–

$T = 149140$ $\quad T^2/N = 9268$

$N = 240$ $\quad \sum x^2 = 90078$

PHASE IV

TABLE IX-f

Insulation Resistance, 27 Weeks After Treatment

Sterilant E - 5% v/v Ethylene imine in Absolute Methanol

Subject r

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	39.5	0.00001	69	55
		-	39.0	0.00001	69	59
1	2	+	36.5	0.1	69	55
		-	36.0*	0.1		
	3	+	32.0	0.1		
		-	29.5*	0.1		
	4	+	30.5	0.1		
		-	29.0*	0.1		
	5	+	18.5	0.1		
		-	17.5*	0.1		
	6	+	20.5	0.1		
		-	19.0*	0.1		
2	2	+	21.5	0.001	69	59
		-	20.5	0.001		
	3	+	53.0	0.001		
		-	48.5	0.001		
	4	+	12.0	0.0001		
		-	9.5	0.0001		
	5	+	3.5	0.0001		
		-	2.5	0.0001		
	6	+	4.5	0.0001		
		-	3.5	0.0001		

*There seemed to be a gradual drift toward zero on the galvanometer scale. Reported reading is maximum before drift became apparent.

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant E - 5% v/v Ethylene imine in Absolute Methanol

Subject s

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	38.0	0.00001	69	59
		-	38.0	0.00001	68	58
1	2	+	1.5	1.0	69	59
		-	0.5			
	3	+	1.5			
		-	1.0			
	4	+	1.5			
		-	0.5			
	5	+	1.0			
		-	1.0			
	6	+	1.0			
		-	1.0			
2	2	+	1.0	1.0	68	58
		-	1.0			
	3	+	1.0			
		-	1.0			
	4	+	1.0			
		-	1.0			
	5	+	1.0			
		-	1.0			
	6	+	1.0			
		-	1.0			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant E - 5% v/v Ethylene imine in Absolute Methanol

Subject t

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+ -	37.5 38.5	0.00001 0.00001	69 68	59 62
1	2	+ -	1.5 0.5	1.0	69	59
	3	+ -	1.0 1.0			
	4	+ -	1.0 1.0			
	5	+ -	1.0 0.5			
	6	+ -	0.5 1.0			
2	2	+ -	1.0 0.5	1.0	68	62
	3	+ -	1.0 1.0			
	4	+ -	1.0 1.0			
	5	+ -	1.0 1.0			
	6	+ -	1.0 1.0			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant F - 5% w/v Formaldehyde in Absolute Methanol

Subject r

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	38.5	0.00001	68	58
		-	39.0	0.00001	69	59
1	2	+	6.5	0.1	68	58
		-	7.0	0.1		
	3	+	9.5	0.1		
		-	8.5	0.1		
	4	+	22.5*	0.1		
		-	24.0*	0.1		
	5	+	5.0	0.1		
		-	5.0	0.1		
	6	+	13.0	0.1		
		-	12.5	0.1		
2	2	+	6.5	0.01	69	59
		-	5.0	0.01		
	3	+	2.0	0.01		
		-	1.0	0.01		
	4	+	3.0	0.001		
		-	1.0	0.001		
	5	+	6.0	0.01		
		-	2.5	0.01		
	6	+	4.5	0.01		
		-	2.5	0.01		

*Galvanometer never came to rest, but varied within ± 2mm of this reading for a short while, then slowly drifted toward zero.

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant F - 5% w/v Formaldehyde in Absolute Methanol

Subject s

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	37.5	0.00001	69	59
		-	38.0	0.00001	68	62
1	2	+	4.5	1.0	69	59
		-	4.5			
	3	+	6.5			
		-	7.0			
	4	+	8.0			
		-	7.0			
	5	+	7.0			
		-	6.5			
	6	+	8.0			
		-	7.0			
2	2	+	2.5	1.0	68	52
		-	2.0			
	3	+	2.0			
		-	2.0			
	4	+	2.5			
		-	2.5			
	5	+	2.0			
		-	2.0			
	6	+	2.5			
		-	2.0			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant F - 5% w/v Formaldehyde in Absolute Methanol

Subject t

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	38.0	0.00001	68	62
		-	37.5	0.00001	68	62
1	2	+	1.0	1.0	68	62
		-	1.0			
	3	+	1.0			
		-	1.0			
	4	+	1.0			
		-	0.5			
	5	+	1.0			
		-	1.0			
	6	+	1.5			
		-	0.5			
2	2	+	1.0	1.0	68	62
		-	0.5			
	3	+	1.0			
		-	0.5			
	4	+	1.0			
		-	0.5			
	5	+	2.0			
		-	1.0			
	6	+	1.0			
		-	0.5			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant G - 5% v/v Beta-propiolactone in Absolute Methanol

Subject r

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	38.0	0.00001	68	62
		-	38.0	0.00001	68	62
1	2	+	3.0	0.0001	68	62
		-	2.5	0.0001		
	3	+	6.0	0.0001		
		-	4.0	0.0001		
	4	+	7.5	0.0001		
		-	5.0	0.0001		
	5	+	1.0	0.0001		
		-	0.5	0.0001		
	6	+	2.5	0.0001		
		-	1.0	0.0001		
2	2	+	10.0	0.1	68	62
		-	10.0	0.1		
	3	+	1.0	0.01		
		-	2.0	0.01		
	4	+	1.0	0.01		
		-	0.5	0.1		
	5	+	7.5	0.1		
		-	7.0	0.1		
	6	+	11.5	0.1		
		-	11.0	0.1		

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant G - 5% v/v Beta-propiolactone in Absolute Methanol

Subject s

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	38.0	0.00001	68	58
		-	38.0	0.00001	68	58
1	2	+	3.0	1.0	68	58
		-	3.0			
	3	+	3.0			
		-	3.0			
	4	+	3.0			
		-	2.0			
	5	+	3.0			
		-	3.0			
	6	+	3.0			
		-	3.0			
2	2	+	3.0	1.0	68	58
		-	2.5			
	3	+	2.0			
		-	2.0			
	4	+	2.5			
		-	2.5			
	5	+	2.0			
		-	3.0			
	6	+	2.0			
		-	3.5			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant G - 5% v/v Beta-propiolactone in Absolute Methanol

Subject t

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	37.5	0.00001	69	59
		-	37.5	0.00001	69	59
1	2	+	1.5	1.0	69	59
		-	0.5			
	3	+	1.5			
		-	1.0			
	4	+	1.5			
		-	1.0			
	5	+	1.5			
		-	1.0			
	6	+	1.5			
		-	1.0			
2	2	+	1.0	1.0	69	59
		-	1.0			
	3	+	1.5			
		-	1.0			
	4	+	1.0			
		-	1.0			
	5	+	1.5			
		-	1.0			
	6	+	1.0			
		-	1.5			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant H - 4.76% w/w Paraformaldehyde in Dow Corning 4 Compound

Subject r

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	37.5	0.00001	68	62
		-	37.5	0.00001	67	66
1	2	+	2.0	1.0	68	62
		-	2.5			
	3	+	2.5			
		-	2.0			
	4	+	2.0			
		-	2.5			
	5	+	2.0			
		-	2.5			
	6	+	2.0			
		-	2.5			
2	2	+	2.0	1.0	67	66
		-	1.5			
	3	+	2.5			
		-	1.5			
	4	+	2.0			
		-	2.0			
	5	+	2.0			
		-	2.0			
	6	+	2.0			
		-	2.0			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant H - 4.76% w/w Paraformaldehyde in Dow Corning 4 Compound

Subject s

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	37.5	0.00001	68	62
		-	37.0	0.00001	68	62
1	2	+	2.5	1.0	68	62
		-	2.0			
	3	+	4.0			
		-	3.0			
	4	+	4.0			
		-	4.0			
	5	+	4.5			
		-	3.5			
	6	+	4.5			
		-	4.0			
2	2	+	3.0	1.0	68	62
		-	2.0			
	3	+	3.0			
		-	2.0			
	4	+	3.0			
		-	2.5			
	5	+	2.5			
		-	2.0			
	6	+	3.0			
		-	3.0			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Sterilant H - 4.76% w/w Paraformaldehyde in Dow Corning 4 Compound

Subject t

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	37.5	0.00001	68	62
		-	37.5	0.00001	68	62
1	2	+	1.5	1.0	68	62
		-	1.0			
	3	+	1.0			
		-	1.0			
	4	+	1.5			
		-	1.0			
	5	+	1.5			
		-	1.0			
	6	+	1.5			
		-	0.5			
2	2	+	1.5	1.0	68	62
		-	1.5			
	3	+	1.0			
		-	1.0			
	4	+	1.0			
		-	1.5			
	5	+	1.5			
		-	1.5			
	6	+	1.0			
		-	1.5			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Controls

Subject r

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	41.5	0.00001	70	59
		-	39.0	0.00001	68	62
1	2	+	1.5	1.0	70	59
		-	2.0			
	3	+	2.0			
		-	1.0			
	4	+	1.5			
		-	1.0			
	5	+	1.5			
		-	1.5			
	6	+	1.5			
		-	2.0			
2	2	+	1.5	1.0	68	62
		-	1.0			
	3	+	1.5			
		-	1.0			
	4	+	1.0			
		-	2.0			
	5	+	1.0			
		-	1.0			
	6	+	1.0			
		-	1.5			

PHASE IV

TABLE IX-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Controls

Subject s

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	38.5	0.00001	68	62
		-	39.0	0.00001	69	63
1	2	+	4.0	1.0	68	62
		-	4.0			
	3	+	6.0			
		-	5.5			
	4	+	4.0			
		-	3.0			
	5	+	4.5			
		-	4.0			
	6	+	5.0			
		-	4.0			
2	2	+	3.0	1.0	69	63
		-	2.0			
	3	+	3.0			
		-	2.5			
	4	+	2.5			
		-	2.0			
	5	+	2.5			
		-	2.5			
	6	+	3.0			
		-	3.0			

PHASE IV

TABLE IX-f (Concluded)

Insulation Resistance, 27 Weeks After Treatment

Controls

Subject t

Replicate	Pin no.	Voltage sign	Galv. defl.	Shunt setting	T, °F	% RH
Short		+	38.0	0.00001	69	63
		-	38.5	0.00001	69	63
1	2	+	1.5	1.0	69	63
		-	0.5			
	3	+	1.0			
		-	0.5			
	4	+	1.0			
		-	0.5			
	5	+	1.0			
		-	1.0			
	6	+	1.0			
		-	1.0			
2	2	+	1.0	1.0	69	63
		-	1.0			
	3	+	1.0			
		-	0.5			
	4	+	1.0			
		-	1.0			
	5	+	1.0			
		-	0.5			
	6	+	1.0			
		-	0.5			

PHASE IV

TABLE X - f

Logarithm of Insulation Resistance 27 Weeks after Treatment, $\log_{10}$ ohms

SUBJECT r						
Replicate	Pin No.	Voltage	Sterilant			
			E	F	G	H
1	2	+	10.03	10.77	8.10	12.27
		-	10.04	10.74	8.18	12.18
	3	+	10.09	10.61	7.79	12.18
		-	10.13	10.66	7.97	12.27
	4	+	10.11	10.23	7.70	12.27
		-	10.13	10.20	7.88	12.18
	5	+	10.33	10.89	8.58	12.27
		-	10.35	10.89	8.88	12.18
	6	+	10.29	10.47	8.18	12.27
		-	10.32	10.49	8.58	12.18
2	2	+	8.26	9.78	10.58	12.27
		-	8.28	9.89	10.58	12.40
	3	+	7.86	10.29	10.58	12.18
		-	7.90	10.59	10.28	12.40
	4	+	7.51	9.11	10.58	12.27
		-	7.60	9.59	11.88	12.27
	5	+	8.06	9.81	10.71	12.27
		-	8.19	10.19	10.73	12.27
	6	+	7.93	9.94	10.52	12.27
		-	8.06	10.19	10.54	12.27
SUBJECT s						
1	2	+	12.40	11.92	12.10	12.18
		-	12.88	11.92	12.10	12.27
	3	+	12.40	11.76	12.10	11.97
		-	12.58	11.73	12.10	12.10
	4	+	12.40	11.67	12.10	11.97
		-	12.88	11.73	12.28	11.97
	5	+	12.58	11.73	12.10	11.92
		-	12.58	11.76	12.10	12.03
	6	+	12.58	11.67	12.10	11.92
		-	12.58	11.73	12.10	11.97
2	2	+	12.58	12.18	12.10	12.09
		-	12.58	12.28	12.18	12.27
	3	+	12.58	12.28	12.28	12.09
		-	12.58	12.28	12.28	12.27
	4	+	12.58	12.18	12.18	12.09
		-	12.58	12.18	12.18	12.17
	5	+	12.58	12.28	12.28	12.17
		-	12.58	12.28	12.10	12.27
	6	+	12.58	12.18	12.28	12.09
		-	12.58	12.28	12.03	12.09

PHASE IV

TABLE X - f (Continued)

Logarithm of Insulation Resistance 27 Weeks after Treatment, $\log_{10}$ ohms

SUBJECT t						
Replicate	Pin No.	Voltage	Sterilant			
			E	F	G	H
1	2	+	12.40	12.58	12.40	12.40
		-	12.88	12.58	12.88	12.57
	3	+	12.57	12.58	12.40	12.57
		-	12.57	12.58	12.57	12.57
	4	+	12.57	12.58	12.40	12.40
		-	12.57	12.88	12.57	12.57
	5	+	12.57	12.58	12.40	12.40
		-	12.88	12.58	12.57	12.57
	6	+	12.88	12.40	12.40	12.40
		-	12.57	12.88	12.57	12.88
2	2	+	12.59	12.57	12.57	12.40
		-	12.89	12.88	12.57	12.40
	3	+	12.59	12.57	12.40	12.57
		-	12.59	12.88	12.57	12.57
	4	+	12.59	12.57	12.57	12.57
		-	12.59	12.88	12.57	12.40
	5	+	12.59	12.14	12.40	12.40
		-	12.59	12.57	12.57	12.40
	6	+	12.59	12.57	12.57	12.57
		-	12.59	12.88	12.40	12.40

PHASE IV

TABLE XI - f

Analysis of Variance in Insulation Resistance, Before and 27 Weeks After Treatment Subject r, s, and t

FACTOR	LEVEL	n_*	T_*	T_*^2/n_*	S_*	df	MS
Subject	r	160	1804.25	69,752	152	2	76
	s	160	1957.38				
	t	160	2018.33				
Replicate	1	240	2884.26	69,600	0	1	0
	2	240	2895.70				
Voltage	+	240	2882.17	69,600	0	1	0
	-	240	2897.79				
Pin No.	2	96	1157.17	69,600	0	4	0
	3	96	1154.34				
	4	96	1154.55				
	5	96	1157.55				
	6	96	1156.35				
Treatment	Before	240	2976.82	69,663	63	1	63
	After	240	2803.14				
Sterilant	E	120	1432.90	69,615	15	3	5
	F	120	1445.47				
	G	120	1422.41				
	H	120	1479.18				
Residuals	-	-	-	-	293	467	1
Total	-	480	5779.96	69,600	524	479	-

$T = 5779.96$ $\quad T^2/N = 69,599$

$N = 240$ $\quad \Sigma x^2 = 70,124$

PHASE IV

TABLE XIII - f

Insulation Resistance, 27 Weeks After Treatment

Subject u and v

Subject	Sterilant	Voltage	Galv. defl.	Shunt Setting	T, °F	%RH
Short			37.5	0.00001	68	62
u		+	3.5	1.0	68	62
		-	2.5			
	F	+	4.5			
		-	4.0			
	G	+	2.5			
		-	1.5			
	H	+	1.5			
		-	2.0			
Control u		+	2.0			
		-	1.0			
v	E	+	4.5	1.0	68	62
		-	4.5			
	F	+	1.5			
		-	1.5			
	G	+	2.0			
		-	1.0			
	H	+	2.0			
		-	2.0			
Control v		+	2.0			
		-	1.0			

PHASE IV

TABLE XIII - f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Subject w

Subject	Sterilant	Voltage	Galv. defl.	Shunt Setting	T, °F	%RH
Short			37.5	0.00001	68	62
w	E	+	3.5	1.0	68	62
		-	2.5			
	F	+	2.5			
		-	2.0			
	G	+	2.0			
		-	1.5			
	H	+	1.5			
		-	1.5			
Control w		+	2.0			
		-	1.0			

PHASE IV

TABLE XIII-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Subject x

Subject	Sterilant	Voltage	Galv. defl.	Shunt Setting	T, °F	% RH
Short			37.5	0.00001	69	63
x, #1	E	+	2.0	1.0	69	63
		-	1.0			
	F	+	1.5			
		-	1.0			
	G	+	1.5			
		-	1.5			
	H	+	2.0			
		-	1.0			
x, #2	E	+	1.5			
		-	1.5			
	F	+	1.0			
		-	1.5			
	G	+	1.5			
		-	1.0			
	H	+	1.5			
		-	1.5			
Control x		+	1.5			
		-	1.5			

PHASE IV

TABLE XIII-f (Continued)

Insulation Resistance, 27 Weeks After Treatment

Subjects z and z'

Subject	Sterilant	Voltage	Galv. defl.	Shunt Setting	T, °F	% RH
Short			37.0	0.00001	68	62
z	E	+	1.0	1.0	68	67
		-	1.5			
	F	+	1.0			
		-	0.5			
	G	+	1.0			
		-	1.0			
	H	+	1.5			
		-	1.0			
Control z		+	1.0			
		-	1.0			
z'	E	+	1.5	1.0	68	67
		-	1.0			
	F	+	1.5			
		-	1.0			
	G	+	2.0			
		-	1.5			
	H	+	2.0			
		-	1.0			
Control z'		+	1.5			
		-	2.0			

PHASE IV

TABLE XIV-f

Insulation Resistance, 27 Weeks After Treatment

Subject y

Sterilant	Pin No.	Voltage	Galv. defl.	Shunt Setting	T, °F	% RH
Short			38.5	0.00001	70	59
E	1-2	+	28.5	1.0	70	59
		-	27.0			
	2-3	+	27.0			
		-	23.5			
	3-4	+	29.0			
		-	28.0			
F	1-2	+	20.0	1.0	70	59
		-	18.5			
	2-3	+	18.0			
		-	16.0			
	3-4	+	19.5			
		-	18.0			
G	1-2	+	18.0	1.0	70	59
		-	17.5			
	2-3	+	15.5			
		-	15.0			
	3-4	+	16.0			
		-	16.0			
H	1-2	+	33.5	1.0	70	59
		-	34.0			
	2-3	+	25.0			
		-	25.0			
	3-4	+	28.5			
		-	24.5			

TABLE XVI - f

Appearance of Subjects r through z', 27 Weeks After Treatment with Sterilant E, 5% v/v Ethylene imine in Methanol

Subject	Replicate	Description
r	1	Insulation spotted, dark deposits on pins, odor.
	2	Same as replicate 1.
s	1	Corroded on outside surfaces, dark deposits on pins, dark specks on insulation, two sides of connector difficult to separate.
	2	Same as replicate 1 except fewer dark specks on insulation.
t	1	Insulation slightly spotted, light-colored deposits on some pins, silver-grey areas on external surfaces, insulation on external wires had green color.
	2	Same as replicate 1.
u		Wood had silver color, mercury present over subject.
v		Wood had silver color, mercury present over subject.
w		Wood had silver color, mercury present over subject.
x	1	Surface spotted.
	2	Same as replicate 1.
y		Surface rough, somewhat etched.
z		No changes in appearance.
z'		No changes in appearance.

TABLE XVI - f (Continued)

Appearance of Subjects r through z', 27 Weeks After Treatment with Sterilant F, 5% w/v Formaldehyde in Absolute Methanol

Subject	Replicate	Description
r	1	Insulation slightly spotted, two sides of connector difficult to separate.
	2	Same as replicate 1.
s	1	No changes in appearance.
	2	No changes in appearance.
t	1	Silver-grey areas on external surfaces, two sides of connector difficult to separate.
	2	No changes in appearance.
u		Wood had silver color, mercury present over subject.
v		Wood had silver color, mercury present over subject.
w		Wood had silver color, mercury present over subject.
x	1	No changes in appearance.
	2	No changes in appearance.
y		Surface rough, somewhat etched.
z		No changes in appearance.
z'		No changes in appearance.

TABLE XVI - f (Continued)

Appearance of Subjects r through z', 27 Weeks After Treatment with Sterilant G, 5% v/v Beta-propiolactone in Methanol

Subject	Replicate	Description
r	1	Insulation spotted, deposits on pins, corroded surfaces, odor, two sides of connector difficult to separate.
	2	Same as replicate 1.
s	1	Corroded on external surfaces, green-blue deposit on pins, powdery deposit on interior of connector, insulation spotted, two sides of connector difficult to separate.
	2	Same as replicate 1.
t	1	Green deposits on pins, silver-grey areas on external surfaces, two sides connector difficult to separate.
	2	Same as replicate 1 except silver-grey areas more extensive, and the two sides not as difficult to separate.
u		Wood had silver color, mercury present over subject.
v		Wood had silver color, mercury present over subject.
w		Wood had silver color, mercury present over subject.
x	1	No changes in appearance.
	2	No changes in appearance.
y		Surface rough, somewhat etched, liquid on the subject.
z		No changes in appearance.
z'		No changes in appearance.

PHASE IV

TABLE XVI - f (Concluded)

Appearance of Subjects r through z', 27 Weeks After Treatment with Sterilant H, 4.76% w/w Paraformaldehyde in Dow-Corning #4 Compound

Subject	Replicate	Description
r	1	Grease on pins and insulation, odor.
	2	Same as replicate 1.
s	1	Grease on pins and insulation.
	2	Same as replicate 1.
t	1	Grease on pins and insulation, silver-grey on external surfaces.
	2	Same as replicate 1.
u		Wood had silver color, mercury present over subject.
v		Wood had silver color, mercury present over subject.
w		Wood had silver color, mercury present over subject.
x ‡	1	No changes in appearance.
	2	No changes in appearance.
y ‡		No changes in appearance.
z		No changes in appearance.
z'		No changes in appearance.

‡ The grease was wiped off these subjects with Kimwipes before the observations and measurements were made.

PHASE IV

TABLE XVII -f

Numbers refer to footnotes on pages 63-69

Subject	Material[1]	Phase	Sterilant			
			Ethylene imine 5% v/v		Formaldehyde 5% w/v	
			in Trichloro-ethylene	in Absolute Methanol	in Methanol 90	in Absolute Methanol
a	Magnesium alloy with Dow 7 Surface treatment	I,II	2			
b	Silicone paint on Magnesium alloy	I	3,92		3	
c	Silicone paint on Magnesium alloy	II	3,92			
d	Gold plate on Magnesium alloy	II			91	
e	Teflon sheet	II				
f	Sheet Stycast	II	43		44	
g	Epoxy-fiberglass block	II				
h	Silicone grease	II	7		8,60	
i	Silicone rubber	II	48		48	
j	Butyl rubber	II	46,49,10		11	
k	Magnesium alloy stainless steel	II II	15,52		16,53	
l	Magnesium alloy stainless steel	II II	19		20	
m	Magnesium alloy stainless steel	II II			23	
n	Aluminum stainless steel silicone rubber	II II II	24		25	
p	Magnesium alloy stainless steel	II II	34		35	
q	Strip coat	II	40,47		41,47	
u	Irradiated polyolefin	IV		68		68
v	Extruded teflon	IV		68		68
w	Teflon wrapping	IV		68		68
x	Teflon sheet	IV		69		
y	Polyurethane potting	IV		70		70
z	Teflon cable covering	IV				
z'	Teflon cable covering	IV				
Bottle	Polyethylene bottle	I	42			

PHASE IV

TABLE XVII-f (continued)

Subject	Material[1]	Phase	Sterilant			
			Beta Propiolactone, 5% v/v			Para formaldehyde 4.76% w/w
			in Distilled Water 33	in Solvent M-17	in Absolute Methanol	in Dow-Corning #4 Compound
a	Magnesium alloy with Dow 7 Surface treatment	I, II	2			
b	Silicone paint on Magnesium alloy	II	3,92	3,92,93		
c	Silicone paint on Magnesium alloy	II	3,92	3,92		
d	Gold plate on magnesium alloy	II	91	92		
e	Teflon Sheet	II	4			
f	Sheet Stycast	II	44	44		
g	Epoxy-fiberglass block	II		5		
h	Silicone Grease	II	6	7		
i	Silicone Rubber	II	45,48	9,48		
j	Butyl Rubber	II	12,49	11,46,49		
k	Magnesium alloy stainless steel	II II	13,50	14,51		
l	Magnesium alloy stainless steel	II II	17	18		
m	Magnesium alloy stainless steel	II II	21	22		
n	Aluminum stainless steel silicone rubber	II II II				
p	Magnesium alloy stainless steel	II II	36	37		
q	Strip coat	II	38,47	39		
u	Irradiated polyolefin	IV			68	68
v	Extruded Teflon	IV			68	68
w	Teflon wrapping	IV			68	68
x	Teflon sheet	IV				75
y	Polyurethane potting	IV			70,74	75
z	Teflon cable covering	IV				
z'	Teflon cable covering	IV				
bottle	Polyethylene bottle	I				

PHASE IV

TABLE XVII-f (continued)

Subject	Material[1] (All from Phase IV)	Sterilants			
		Ethylene imine 5% v/v		Formaldehyde 5% w/v	
		in Trichloro-ethylene	in Absolute Methanol	in Methanol 90	in Absolute Methanol
O Cannon	Appearance Resistance Contact Insulation	56,62		63	
O' Bendix	Appearance Resistance Contact Insulation	30 58,64		31,32 59,64	
r Bendix	Appearance Resistance Contact Insulation		66 85		72 86
s Deutsch	Appearance Resistance Contact Insulation		67 65,83		82
t Cannon	Appearance Resistance Contact Insulation		71 88		73 89

PHASE IV

TABLE XVII-f (continued)

Subject	Material[1] (All from Phase IV)	Sterilants			
		Beta-Propiolactone .5% v/v			Paraformaldehyde 4.76% w/w
		in Distilled Water 33	in Solvent M-17	in Absolute Methanol	in Dow Corning #4 Compound
O Cannon	Appearance		26		
	Resistance				
	Contact	54, 61	55, 63		
	Insulation				
O' Bendix	Appearance	28, 29	27		
	Resistance				
	Contact				
	Insulation	57, 64	58, 64		
r Bendix	Appearance			66, 76	79
	Resistance				
	Contact				
	Insulation			87	
s Deutsch	Appearance			77	80
	Resistance				
	Contact			83	84
	Insulation				
t Cannon	Appearance			78	80,81
	Resistance				
	Contact			88	
	Insulation				

PHASE IV

TABLE XVII - f (Continued)

Footnotes to TABLE I

1. A complete description of the materials in each of the subjects is presented on page 33 of reference 1 and on page 69 of reference 2.
2. Glossy spots and discoloration were left on the surface after the sterilant was evaporated.
3. Some of the paint came off the specimen.
4. Small brown spots appeared when the sterilant evaporated.
5. There was a light film on the shiny surface and the epoxy was attacked when the sterilant evaporated.
6. The residue was oily fluid even after it was dried over Drierite for 48 hours.
7. The residue was greasy.
8. The residue was a hard white deposit with the odor of formaldehyde.
9. The subject had a dirty appearance after the sterilant had evaporated.
10. Light yellow-brown deposits on the surface appeared.
11. Film on the surface appeared.
12. Small deposits on the surface after the sterilant evaporated.
13. The surface was corroded.
14. The surface was dulled; there were deposits; and the mating surfaces adhered together.
15. There were white deposits on the subject.
16. There was a heavy deposit of paraformaldehyde on all occluded surfaces.
17. The outside was corroded as were the flat mating surfaces.

PHASE IV

TABLE XVII - f (Continued)

18. The surface was dull after the sterilant evaporated.
19. There was a filmy deposit over the subject. The mating surfaces were shiny.
20. A surface film appeared and there were deposits on the occluded surfaces after the evaporation of the sterilant.
21. There were glossy spots and brown spots on the surfaces after the sterilant evaporated.
22. There was a gummy deposit on an occluded surface.
23. There were small deposits and pits on one of the replicates.
24. There was a small amount of dark substance on the threads under the nut.
25. There were deposits on the threads of the screw.
26. There was a gummy deposit on all surfaces after the sterilant evaporated.
27. There was a gummy deposit on all surfaces after the sterilant evaporated.
28. The housing was spotted.
29. There were yellow deposits on wet insulation.
30. The entire connector appeared oily.
31. There was a white film on the body.
32. The insulation around the pins was wet.
33. Sparkletts "Distilled Water" (actually deionized water) was used.
34. The surface was dulled slightly, pitted with corrosion, and white deposits were present.

PHASE IV

TABLE XVII - f (Continued)

35. There were white crystalline deposits.

36. The subject was corroded except for the nut and threads covered by the nut.

37. There was a light film on the surfaces.

38. The coating was slightly blistered.

39. The coating was tacky and wrinkled where it had been lying in solvent against a glass surface.

40. The coating was stuck to the dish where exposure to the solvent was the greatest.

41. The coating dissolved and recongealed.

42. Pure ethylene imine dissolved a hole right through the polyethylene bottle in which it was stored.

43. The subject gained about 1% in weight on exposure to the sterilant.

44. The subject gained about 1/2 of 1% in weight on exposure to the sterilant.

45. One of the two replicates seemed to gain 3% in weight while the other one gained only 0.03% in weight on exposure to the sterilant.

46. The subject appeared to gain about 1/2 of 1% in weight on exposure to the sterilant.

47. The subject seemed to lose about 1/2% of its weight on exposure to sterilant This 1/2 of 1% included not only the weight of the strip coat but also of the aluminum strip on which the strip coat was deposited. Thus, the weight lost was larger than 1/2 of 1%.

PHASE IV

TABLE XVII - f (Continued)

48. The subject seemed to increase in linear dimensions on the order of 1-2% on exposure to the sterilant.

49. The subject seemed to increase in linear dimensions on the order of 1/2% on exposure to the sterilant

50. Subject k's contact resistance seemed to increase between 5 and 30% on exposure to the sterilant where as for subject k there was an approximate 5% decrease in contact resistance.

51. For subject k there was a change in contact resistance up to 23% where as for subject k' there was a decrease in contact resistance between 21 and 63%.

52. For subject k there was an increase in contact resistance on the order of 35% and for subject k' one of the replicates reduced in contact resistance by 41% where as the other increased in contact resistance by almost 70%.

53. The contact resistance of subject k increased between 70 and 140% where as that of subject k' decreased on the order of 40%.

54. The increase in contact resistance for subject O was on the order of 10 to 30%.

55. The contact resistance decreased as much as 60%.

56. The contact resistance of the individual pins increased 12 to 209%.

57. The log of the resistance of the insulation dropped in some cases to as low as 5 after treatment with the sterilant.

PHASE IV

TABLE XVII - f (Continued)

58. The log resistance of the insulation dropped from 12 to 10 after exposure to the sterilant.

59. The log resistance of the insulation dropped from 12 to 7 after exposure to the sterilant.

60. Less than 5% of the specimen dropped in the sterilant dissolved whereas for the other sterilants, up to half of each specimen dissolved.

61. After 4 months of storage in contact with the sterilant, the contact resistance of one of the pins increased by over 4000%. The resistance of the other pins increased by no more than 100%. The high resistance pins appeared to have been corroded.

62. The resistance of two pins increased to as high as 800% over the initial value, the others appeared to remain unchanged from the earlier post-treatment values.

63. The contact resistance during storage seemed both to increase and decrease in no particularly predictable manner. The change in contact resistance was less than 50% however.

64. For each of the sterilants the insulation resistance increased during the storage period. For beta-propiolactone in distilled water the final value was less than it was for the other sterilants.

65. The range of variability of the contact resistance was $\pm$ 50%.

66. An odor was noticed, there were dark deposits on the pins, and the insulation was spotted.

PHASE IV

TABLE XVII - f (Continued)

67. The outside surfaces were corroded, dark deposits were on the pins, and dark specks were on the insulation. The two sides of the connector were difficult to separate.

68. The wood had a silver color, mercury was present over the subject.

69. The surface was spotted.

70. The surface was rough and somewhat etched.

71. The insulation was slightly spotted; there was a light-colored deposit on some pins; silver-grey areas on the external surfaces; and the insulation of the external wires had a green color.

72. The insulation was slightly spotted and the two sides of the connector were difficult to separate.

73. There were silver-grey areas on the external surfaces and the two sides of the connector were difficult to separate.

74. There was liquid on the subject. The surface was somewhat etched.

75. The grease was removed before the observations and measurements were made.

76. The surfaces were corroded and the connector was difficult to separate.

77. There was corrosion on the external surfaces, green-blue deposits on the pins, and powdery deposits in the interior of the connector; the insulation was spotted, and the two sides of the connector were difficult to separate.

PHASE IV

TABLE XVII - f (Concluded)

78. There were green-blue deposits on the pins, silver-grey areas on the external surfaces, and the connector was difficult to separate.

79. There was an odor. Grease was on the pins and the insulation.

80. There was grease on the pins and insulation.

81. There were silver-grey areas on the external surfaces.

82. The controls showed a -20 to a +38% change in the contact resistance.

83. There were changes up to 7251 in the contact resistance.

84. There was a variability of change from a -51% to a +85% in contact resistance

85. The log resistance of the insulation dropped from 12.22 to 7.51 after exposure to the sterilant.

86. The log resistance of the insulation dropped from 12.34 to 9.11 after exposure to the sterilant.

87. The log resistance of the insulation dropped from 12.22 to 7.70 after exposure to the sterilant.

88. There were changes up to 8744% in the contact resistance.

89. There were changes up to 352% in the contact resistance.

90. Prepared by diluting formalin solution in absolute methanol.

91. Edges were corroded. There were many surface stains.

92. Surface stains were present after sterilant evaporated.

93. Surface was tacky.

PHASE IV

TABLE XVII - f

Materials Expected to be Found in the Components of Electrical Connectors

A. BODY SHELLS

	Material	Finish
1)	Nickel-iron alloy	Gold plate, 0.00001" min. per MIL-G-45204
2)	Steel per QQ-S-636 (subjects o and t)	Cadmium plated per QQ-P-416a Type II class 2 with yellow chromate supplementary coating.
3)	Brass per QQ-B-626, Composition 22 half hard	Gold plate, 0.00003" min. per MIL-G-45204 over silver plate 0.0002" min.
4)	Brass per QQ-B-613, Composition 11 half hard	Gold plate, 0.00003" min. per MIL-G-45204 over silver plate, 0.0002" min.
5)	Aluminum Alloy per QQ-A-351, Condition T.	Anodize per AMS-2468, 0.002" min. thick.
6)	Aluminum Alloy per QQ-A-268 including Condition T.	Silver plate, 0.0002" min. per QQ-S-365 plus rhodium flask.
7)	Aluminum Alloy per QQ-A-270	Gray anodize per MIL-A-8625, Type I
8)	Aluminum Alloy, machined bar stock (subjects o' and r)	Cadmium plated to QQ-P-416, olive drab chromate after finish.
9)	Unknown (subject s)	Unknown

PHASE IV

TABLE XVII - f (Continued)

	Material	Finish
B.	CONTACTS	
1)	Nickel-iron alloy	Gold plate, 0.0001" min. per MIL-G-45204
2)	Brass per QQ-B-626, Composition 22, half hard	Gold plate, 0.0001" min. per MIL-G-45204 Type I, Class 2
3)	Brass per QQ-B-626, Composition 22, half hard	Gold plate, 0.00003" min. per MIL-G-45204 over silver plate, 0.0002" min.
4)	Coin silver per MIL-S-13282, hard temper	Gold plate, 0.00003" min. per MIL-G-45204 over silver plate, 0.0002" min.
5)	Coin silver, Grade C, per MIL-S-13282, hard temper	Gold plate 0.00003" min. per MIL-G-45204 Type II.
6)	Beryllium Copper per QQ-C-530	Gold plate 0.00005" min. per MIL-G-45204 over silver plate, 0.0002" min. per QQ-S-365.
7)	Copper Alloy (subjects o, r, and s)	Gold plate 0.00003" min. over silver plate.
8)	Phosphor bronze per ASTM B139 Alloy B2 (subjects o and t)	Gold plate 0.00003" min. per MIL-G-45204 over silver plate 0.0002" min. per QQ-S-3652.
9)	Brass per QQ-B-626a, Composition 22 (subjects o and t)	Gold plate 0.00003" min. over silver plate 0.0002" min.
C.	INSULATION INSERTS	
1)	Glass	
2)	Silicone Rubber	
3)	Teflon per MIL-P-19468	

PHASE IV

TABLE XVII - f (Concluded)

C.	INSULATION INSERTS (Continued)
4)	Diallyl phthalate per MIL-M-14 Type SDG
5)	Diallyl phthalate per MIL-M-19833 Type GDI-30 (subject o and part of t)
6)	MIL-STD-417 Grade SC 715 $A_1B_1E_3$ 75 Durometer (subjects o' and r)
7)	Unknown (subject s and Cinch part of t)
D.	LESS CRITICAL COMPONENTS
1)	Silicone rubber per MIL-R-5847 (Class II included)
2)	Teflon per MIL-P-22296
3)	Black iridite over anodizing on alloy steel B1113.
4)	Phosphor bronze per QQ-P-330, Composition A with anodizing per AMS-2468 0.002" thick.

IV. PHASE V

A. PREPARATION OF SPORE SUSPENSIONS

Spore suspensions of B. subtilis, var. niger (JPL origin), Cl. sporogenes (ATCC No. 7955), and Trichoderma sp. (ATCC No. 9645) were assayed for viable cell content. The suspensions of Cl. sporogenes and Trichoderma sp. were those prepared for previous work on this contract, and they have been under refrigeration continuously since then. The B. subtilis, var. niger suspensions were those prepared earlier for use in Phase II, and they have likewise been under continuous refrigeration. The three suspenisons of each organism were found to contain 10^{10} 10^{9}, and 10^{8} cells/ml.

B. PREPARATION OF SPECIMENS

The spore inoculums, which were exposed to various desiccating conditions with the experimental plan described in Table I, resided on flat glass beads attached to a solid glass rod.

They had been deposited from suspension in 0.01 ml of distilled water. They were dried in air at room temperature. The rods with the attached beads (trees) were designed to fit inside glass tubes, 1 inch in diameter, attached to the vacuum manifold. All the inoculums on any particular tree represented the same species of organism. Different levels of inoculum were, however, represented on each tree.

C. EXPOSURE OF SPORES TO DESICCATING CONDITIONS

The trees which were to be subjected to vacuum were all inoculated on the same day and placed in the glass tubes in a vertical position with the connection to the vacuum pump at the bottom of the tube. The tubes, six in number, were four feet long and contained a total volume of 2.8 liters. After the trees were in place, the tubes were sealed with a torch. The vacuum of 2×10^{-5} Torr. was produced by a mercury diffusion pump separated from the trees by a liquid nitrogen trap. The vacuum of 1×10^{-8} (nominal) was produced by a Varian eight-liters-per-minute Vac Ion pump. Three days later the trees, which were to be subjected to atmospheric pressure, 50% relative humidity treatment, were inoculated and stored on clean brown wrapping paper in an air-conditioned room. On the day following this, the 2×10^{-5} Torr. vacuum was broken because the tree containing the Trichoderma sp. spores had lost many of its beads. After the tree was replaced, the vacuum was re-established.

Even though the spores were dried at room temperature on the glass trees, there was enough water vapor released into the tubes to keep the pressure above 1×10^{-8} for several days. Four days after starting the Vac Ion pump, the pump pressure was at 9×10^{-8} Torr., and two days later a pressure of 4×10^{-8} Torr. was reached. The measurement of length of exposure started on the fifth day. By the twelfth day, the pressure was at 1×10^{-8} Torr., where it remained for the remaining period of exposure. On the twenty-first day after the measurement of the length of exposure was begun, the Vac Ion pump was turned off. The final pressure in the tubes was established by permitting the pressure in the tubes to build up pressure in the pump while

the pump was off. After one-half hour of pressure equilibration the pump was turned on again and the pump pressure noted. The one-half hour of pressure equilization did not change the pressure at the pump. After the pressure was measured, the 10^{-5} and the 10^{-8} Torr. manifolds were opened and the trees removed.

On the same day all the trees were placed on a piece of clean brown wrapping paper on a table where the beads were plucked from the rods using sterile forceps. The beads were placed in sterile beakers. These beakers were then transferred to a sterile dry box* for the application of the liquid sterilants.

D. STERILIZATION MEASUREMENTS

The one-ninth fractional factorial experiment described in Table II shows the combinations of the five variables actually studied. The results obtained for the three different species of microorganisms have been summarized in Table I. Table II presents in another manner the nature of the effect of the five variables on the inoculum viability.

The beads taken from the trees were subjected to the different levels of concentration and length of exposure to sterilants while lying in sterile Petri dishes in the dry box. By spraying, the beads were covered with the different sterilants. After the predetermined exposures were completed, the beads were taken from the dishes and dropped each into its tube of appropriate nutrient medium. These tubes were then placed in the proper environment

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* a box continuously supplied with sterile air and irradiated with ultraviolet, except when specimens were in the box.

for viable cells to proliferate. The B. subtilis, var. niger in Trypticase soy broth+ and the Cl. sporogenes in fluid thioglycollate were incubated at 37°C. The mold in Sabouraud's broth was incubated at room temperature. A total of eleven beads at each level of inoculum were prepared for this experiment, wherever the total number of beads permitted it to be done. Eight beads were used to measure the effect of the sterilants; three were used for controls.

Two types of controls were used in these experiments. One control consisted of dropping two of the inoculated beads, before any sterilizing treatment, into two tubes of the nutrient broth to determine whether the vacuum conditions killed the cells or changed their growth characteristics. The other control was designed to measure the extent of bacteriostatic action by the sterilant and the nutrient value of the broth. After the bacteriostasis control bead had been treated with sterilant and dropped into the broth, then 100 viable spores of the appropriate microorganism were introduced into that same tube of broth. If the broth used to detect viable organisms in the treated specimens were suitable, the bacteriostasis control broth should have become turbid with growing cells.

Examination of the data summarized in Table II confirms the validity of the experimental data. Table III presents an analysis of variance for the results of Table II.

+ the Baltimore Biological Laboratories supplied all the medium ingredients in the form of dry powders.

The procedures[1] for calculating the entries in the analysis of variance table are similar to those used in the earlier phases of the study. The factors which had the most significant effect on the sterilization efficacy are those with the largest values of mean square (MS). As in the experiment described in the Semifinal Report, the concentration and length of exposure have greater effects than do the sterilant and the vacuum exposure. In the earlier study, a valid assessment of the variation in response of the several organisms was not possible. In the present study the mold spores were much less resistant to the sterilants than were spores of B. subtilis, var. niger. The organisms seemed to resist the exposure to vacuum in the same order although the observations of the effect of vacuum on viability were too few to permit firm conclusions to be drawn.

The effect of vacuum exposure on the resistances of the spores to chemical sterilants has not been established by the results reported in Tables I and II. The lack of viable cells on the Cl. sporogenes and Trichoderma sp. treated specimens could have been the result of the destructive effect of the vacuum alone. The data do not indicate a large increase in resistance to chemical sterilants as a result of vacuum exposure, though the B. subtilis, var. niger data do indicate a moderate increase in resistance may occur.

The information in Table III is useful in interpreting the analysis of variance because the use of a fractional factorial design confounds main effects with the indicated two-factor interactions. Because the two-factor interactions AB, AC, BD, and CD are both small and free of two-factor aliases, their MS values may be assumed to be due to residual random variation.

Lumping these together produces a residual MS of 20.4 with 8 degrees of freedom. Calculation of F shows that at the 99% confidence level the length of exposure to the sterilant and the type of organism have a highly significant effect on sterilization effectiveness, as might be expected. At the 95% confidence level, the concentration of the toxic chemical in the sterilant is also significant. It is interesting to note, too, that the combined effects of organims and toxic chemical are more significant than either of them alone. B. subtilis, var. niger appears to be least resistant to ethylene imine, Cl. sporogenes appears to be least resistant to beta-propiolactone, and Trichoderma sp. appears not to be resistant to any of the sterilants.

The precision in the information gained in Phase V could be increased by performing a second fraction of the factorial experiment with these same five factors. The usefulness of such an experiment is not apparent, however. The experiment performed confirmed, with sufficient confidence, that to achieve sterility, the sterilant must be designed to kill the most resistant organism, and that concentration of toxic chemical and exposure must be maximum. The experiment indicated that vacuum exposure, like solid encasement may make spores refractory to chemical sterilants.

E. REFERENCES

1. Connor, W. S. and M. Zelen. "Fractional Factorial Experiment Designs for Factors at Three Levels," National Bureau of Standards Applied Mathematics Series'. 54, Washington, D. C.: Government Printing Office, (May 1, 1959).

TABLE I-f

1/9 Fractional Factorial Design of 5 Variables
Positive Tubes *

		A_0									A_1									A_2								
		B_0			B_1			B_2			B_0			B_1			B_2			B_0			B_1			B_2		
		C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2
D_0	E_0	24					10		22																			
	E_1											0		1					0									
	E_2																					0		0		2		
D_1	E_0																			24					0		6	
	E1		20		18					0																		
	E_2												0		0		4											
D_2	E_0										24					1		21										
	E_1																				23		24					0
	E_2			0		0		8																				

*After 14 days incubation at the appropriate temperature.

PHASE V

TABLE I - f (Concluded)

A	=	Sterilant	A_0	=	Formaldehyde
			A_1	=	Beta-propiolactone
			A_2	=	Ethylene imine
B	=	Concentration of Sterilant	B_0	=	0.2%
			B_1	=	1%
			B_2	=	5%
C	=	Time Sterilant is Applied	C_0	=	5 sec
			C_1	=	5 min
			C_2	=	5 hours
D	=	Environmental Conditions	D_0	=	10^{-8} mm Hg
			D_1	=	2×10^{-5} mm Hg
			D_2	=	50% Humidity; Atmospheric pressure
E		Organism	E_0	=	B. subtilis, var. niger
			E_1	=	Cl. sporogenes
			E_2	=	Trichoderma sp.

PHASE V

TABLE II

Results of Fractional Factorial Design

Organism, B. subtilis, var. niger

Sterilant in methanol	Pressure, Torr.	Time of application	Concentration %	Log_{10} of inoculum	Number of positive tubes	Bacteriostasis control	Growth control
Formaldehyde	1×10^{-8}	5 sec.	0.2	8	8	+	+
				7	8	+	+
				6	8	+	+
		5 hours	1.0	8	5	+	
				7	3	+	
				6	2	+	
		5 min.	5.0	8	8	+	
				7	6	+	
				6	8	+	
Ethylene imine	2×10^{-5}	5 sec.	0.2	8	8	+	+
				7	8	+	+
				6	8	+	+
		5 hours	1.0	8	0	+	
				7	0	+	
				6	0	+	
		5 min.	5.0	8	1	+	
				7	3	+	
				6	2	+	

PHASE V

TABLE II (Continued)

Results of Fractional Factorial Design

Organism, B. subtilis, var. niger

Sterilant in methanol	Pressure Torr.	Time of application	Concentration %	Log_{10} of inoculum	Number of positive tubes	Bacteriostasis control	Growth control
Beta-propiolac-tone	7.6×10^2	5 sec.	0.2	8	8	+	+
				7	8	+	+
				6	8	+	+
		5 hours	1.0	8	0	+	
				7	0	+	
				6	1	+	
		5 min.	5.0	8	8	+	
				7	8	+	
				6	5	+	

TABLE II (Continued)

Results of Fractional Factorial Design

Organism, Cl. sporogenes

Sterilant in methanol	Pressure, Torr.	Time of application	Concentration %	Log_{10} of inoculum	Number of positive tubes	Bacteriostasis control	Growth control
Formaldehyde	2×10^{-5}	5 min.	0.2	8	8	+	+
				7	6	+	+
				6	6	+	+
		5 sec.	1.0	8	8	+	
				7	7	+	
				6	3	+	
		5 hours	5.0	8	0	+	
				7	0	+	
				6	0	-	
Ethylene imine	7.6×10^{2}	5 min.	0.2	8	8	+	+
				7	7	+	+
				6	8	+	+
		5 sec.	1.0	8	8	+	
				7	8	+	
				6	8	+	
		5 hours	5.0	8	0	+	
				7	0	+	
				6	0	+	

PHASE V

TABLE II (Continued)

Results of Fractional Factorial Design

Organism, Cl. sporogenes

Sterilant in methanol	Pressure, Torr.	Time of application	Concentration %	Log_{10} of inoculum	Number of positive tubes	Bacteriostasis control	Growth control
Beta-propio-lactone	10^{-8}	5 min.	0.2	8	0	+	-
				7	0	+	-
				6	0	+	-
		5 sec.	1.0	8	1	+	
				7	0	+	
				6	0	+	
		5 hours	5.0	8	0	+	
				7	0	+	
				6	0	+	

PHASE V

TABLE II (Continued)

Results of Fractional Factorial Design

Organism, Trichoderma sp.

Sterilant in methanol	Pressure, Torr.	Time of application	Concentration %	Log_{10} of inoculum	Number of positive tubes	Bacteriostasis control	Growth control
Formalde-hyde	7.6×10^{2}	5 hours	0.2	8	0	+	+
				7	0	+	+
				6	0	+	-
		5 min.	1.0	8	0	+	
				7	0	+	
				6	0	+	
		5 sec.	5.0	8	8	+	
				7	0	+	
				6	0	+	
Ethylene imine	10^{-8}	5 hours	0.2	8	0	+	-
				7	0	+	-
				6	0	-	-
		5 min.	1.0	8	0	+	
				7	0	+	
				6	0	+	
		5 sec.	5.0	8	0	+	
				7	0	+	
				0	2*	+	

TABLE II (Concluded)

Results of Fractional Factorial Design

Organism, Trichoderma sp.

Sterilant in methanol	Pressure Torr.	Time of application	Concentration %	Log_{10} of inoculum	Number of positive tubes	Bacteriostasis control	Growth control
Beta-propio-lactone	2×10^{-5}	5 hours	0.2	8	0§	+	+
				7	0	-	+
				6	0	+	+
		5 min.	1.0	8	0§	+	
				7	0	+	
				6	0	+	
		5 sec.	5.0	8	4§	+	
				7	0	+	
				6	0	-	

* Possible contaminants

§ Only four tubes were prepared

\+ The media in the tubes became turbid with proliferating cells.

\- The media in the tube remained clear after incubation.

PHASE V

TABLE III - f

Aliases for the Fractional Factorial Design[1]

FACTORS: A, B, C, D, E.

I = ABCDE

BLOCK CONFOUNDING: A B^2 C^2 D

A = DE^2

B = CE^2

C = BE^2

D = AE^2

E = AD = BC

AB = no other two factor interactions

AC = no other two factor interactions

AD = BC = E

AE = DE = AD^2

BC = AD = E

BD = no other two factor interactions

BE = CE = BC^2

CD = no other two factor interactions

CE = BE = BC^2

DE = AE = AD^2

AB^2 = CD^2

AC^2 = BD^2

PHASE V

TABLE IV - f

Analysis of Variance in Sterilization Effectiveness

Factor	Level		n_*	T_*	$\sum \frac{T_*^2}{n_*}$	S	df	MS
Main Effects	A	A_0	9	102	2138	145	2	72.5
		A_1	9	51				
		A_2	9	79				
	B	B_0	9	115	2234	241	2	120.5
		B_1	9	54				
		B_2	9	63				
	C	C_0	9	129	2803	810	2	405
		C_1	9	92				
		C_2	9	11				
	D	D_0	9	59	2096	103	2	51.5
		D_1	9	72				
		D_2	9	101				
	E	E_0	9	132	2780	787	2	393.5
		E_1	9	86				
		E_2	9	14				
Interactions	AB	AB_0	9	93	2054	61	2	30.5
		AB_1	9	60				
		AB_2	9	79				
	AC	AC_0	9	80	2000	7	2	3.5
		AC_1	9	71				
		AC_2	9	81				
	AE	AE_0	9	107	2263	270	2	135
		AE_1	9	86				
		AE_2	9	39				

TABLE IV - f (Concluded)

Analysis of Variance in Sterilization Effectiveness

Factor	Level		n_*	T_*	$\sum \frac{T_*^2}{n_*}$	S	df	MS
Interactions	BD	BD_0	9	59	2051	58	2	29
		BD_0^1	9	84				
		BD_2^1	9	89				
	BE	BE_0	9	72	2030	37	2	18.5
		BE_0^1	9	68				
		BE_2^1	9	92				
	CD	CD_0	9	71	2030	37	2	18.5
		CD_0^1	9	69				
		CD_2^1	9	92				
	AB^2	$(AB^2)_0$	9	54	2111	118	2	59
		$(AB^2)_1$	9	78				
		$(AB^2)_2$	9	100				
	AC^2	$(AC^2)_0$	9	71	2035	42	2	21
		$(AC^2)_1$	9	68				
		$(AC^2)_2$	9	93				
Total			27	232	1993	2711	26	-

$T = 232$

$N = 27$

$\frac{T^2}{N} = 1993$

$\sum x^2 = 4704$

V. CORRECTIONS IN ALL PREVIOUS REPORTS ON EVALUATION OF LIQUID STERILANTS

A. Opfell, J.B., C.E. Miller, and P.N. Hammons "Evaluation of Liquid Sterilants, Phases I and II," Final Report on Jet Propulsion Laboratory Contract No. N1-143452, South Pasadena, California: Dynamic Science Corporation, (August 28, 1962)

1. TABLE OF CONTENTS:

III, A.2.f.,"Stippable" should be "Strippable"

III, B.1,"Phase II" should be "Phase II'"

"III, REFERENCES" should be "IV REFERENCES"

There is no page number.

2. TEXT:

Page 4, line 4, "soley" should be "solely"

Page 10, line 24, "milliliter" should be "milliliters"

Page 17, line 2, "number" should be "numbers"

Page 17, line 3, "is" should be "are"

Page 19, line 8, delete "measurements of"

Page 19, line 15, change to "well as for each of all"

Page 20, line 14, "reaction" should be "reactions"

Page 30, line 23, change to "dishes and put into individual"

Page 30, line 13, change to "solid phase (5)"

Page 31, line 24, "proposal" should be "report"

Page 32, line 16, "one milliliters" should be "0.2 milliliters"

Page 35, line 33, "DOM 508NM1" should be "DDM 50S NM1" and "DOM 50P NM1" should be "DDM 50P NM1"

Page 40, lower half of page, "DOM-505-NM-1" and "DOM-50P-NM-1" should read "DDM 50S NM1" and "DDM 50P NM1" respectively.

Page 49, line 12, "Table IV" should be "Table IX"

Page 55, line 16, "that an error" should be "of an error"

Page 56, line 5, "as those" should be "than those"

Page 60, add footnote "The terminology 1:10 means that no dilution of the rinse water was made. It means 1 in 1 rather than 1 to 1 dilution. A 1 to 1 dilution would be written 1:2."

Page 63, add reference: (5) Maass, O. and E. Boomer. J. Am. Chem. Soc., 44, 1709 (1922)

3. TABLES AND FIGURES

Page 3, line 4, "Bacteriostatis" should be "bacteriostasis"

Page 7, column 5, "n" should be "n_*"

Page 11, line 2, "stranis" should be "strains" and third vertical line should extend to top line of the table

Page 14, delete "0" on extreme right side of table

"In some cases" should be "*In some cases".

Page 15, "2% $^{v}/_{v}$ should be "2% $^{w}/_{v}$"

Page 16, column 4 "143^{1}" should be "143^{2}"

Page 26, Next to last line "o' -4--" should be "o'-3--"

Page 27, "*k' was exposed" should be " * k' was exposed"

Page 28, replace entire page with the new page which follows.

Page 30, column 12, replace the last entries "1,0,76,123" with "1,76,0,123"

Page 30, column 13, move entries "40, 5, 58, 4" to column 14.

Page 33, replace entire page with the new page which follows

Page 34, "o" should be "o'"

Page 39, last two lines, "0.097" and "1.47" should be "-0.097" and "-1.47"

Page 40, replace the entire page with the new page which follows.

Page 41, column 9, "7.6" and "8.4" should be "-7.6" and "-8.4"

Page 41, under both columns headed "Change, percent" the seventh and eighth entries should read "0.0", under the second column headed "Before Treatment" delete all asterisks.

Page 43, last column, "4.17" should be "2.46"

Page 44, last column, "1.57" should be "3.60"

Page 45, last column, "-6.0" should be "6.0"

Page 48, last column, "58.0" and "64.1" should be "70.3" and "76.9"

Page 49, replace the entire page with the new page which follows.

Page 50, columns 6 and 11 on all entries, move decimal points three places to the left.

Page 51, footnotes, "n" should be "N" and "Table" should be "Table XIV"

Page 52, first entry under column "1" should be "86"

Page 55, column 5, "0.0001" should be "0.00001"

Page 55, column 7, "56" should be "59"

Page 61, extend fourth vertical line to separate "Galv." and "Shunt"

Page 64, footnotes, "n" should be "N"

Page 65, last column, "42.8" should be "49.4"

Page 66, replace the entire page with the new page which follows

Page 72, column headings, delete all "1:"

Page 73, column headings, delete all "1:"

extend third vertical line to separate "Strip" and "Plate"

Page 74, change "Gags" to "Bags"

B. Opfell, J.B., C.E. Miller, and A.L. Louderback, "Evaluation of Liquid Sterilants", Semifinal Report on Jet Propulsion Laboratory Contract No. N2-150247, South Pasadena, California: Dynamic Science Corporation (March 16, 1962)

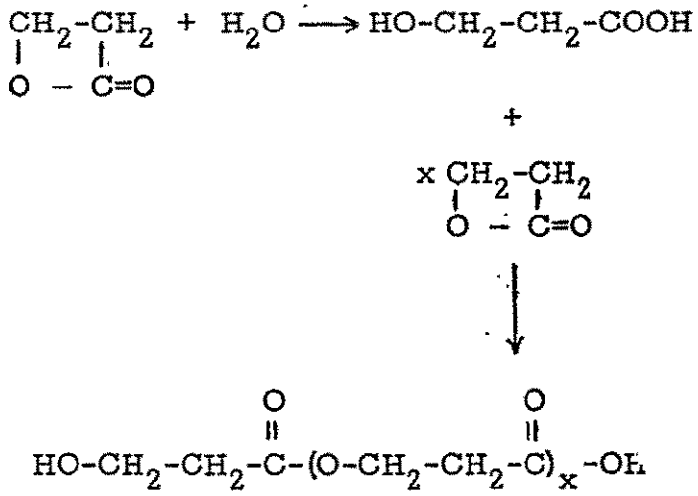
1. TEXT:

Page 3, line 13, "and ether" should be "and ether at room temperature"

Page 5, "paragraph d" should be "paragraph 2d"

Page 19, line 11, "appears" should be "disappears"

Page 24, equation: The equation should read:



Page 33 "21.99, 43.65, and 53.73" should be "22.98, 45.96, and 57.45"

Page 38, line 6, "ablve" should be "above"

Page 40, line 5, "4.69%" should be "4.76%"

Page 40, last line, "10^6, 10^7, and 10^8" should be "10^8, 10^9, and 10^{10},

Page 41, lines 3 and 20, "4.69%" should be "4.76%"

Page 42, line 4, "4.69%" should be "4.76%"

Page 51, column 2, "4.69%" should be "4.76%" in each of three entries.

Page 51, footnote II "0.01" should be "0.001"

Page 52, column 1, "4.69%" should be "4.76%"

Page 52, footnote +, "0.01" should be "0.001"

Page 69, lines 8,9,16 and 17, "DOM" should be "DDM"

Page 110, place vertical line through table to separate "Replicate" and "Pin No."

Pages 113, 114, 115, 116, 117, and 118, title "4.69%" should be "4.76%$^{W}/_{w}$" and "#4" should be "#4 compound"

Page 121, row 8, "372" and "0.80" should be "371" and "0.79" respectively

Page 122, "4.69%" should be "4.76%" and "#4" should be "#4 compound"

Page 135, line 5, "one piece" should be "on a piece"

Page 135, line 12, "actual" should be " actually"

Page 135, line 17, "length" should be "lengths"

Page 138, line 9, "concentration and " should be "concentration of sterilant and "

Page 139, Replace entire page with the new page which follows

Page 147, in square, reverse the entries "21" and "22"

Page 148, replace entire page with the new page which follows

Page 155, line 9, "LD_{50}" should be "LD_{50}*"

Page 155, add footnote: "LD_{50} is the dose which, if administered to a large number of animals, would be lethal to half of them, on the average."

Page 176, reference 12, "9:" should be "9:"

Page 176, reference 13, "AM. Indust. Hyg. Assn. Quart." should be Am. Indust. Hyg. Assn. Quart."

PHASE II

TABLE V

Analysis of Variance in the Number of Contaminants

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS
Subject	a	4	2				
	e	4	0				
	f	4	14				
	g	4	1				
	h	4	4				
	i	4	5				
	j	4	16				
	k	4	5				
	l	4	0				
	m	4	5				
	n	4	4				
	o	4	6				
	o'	4	9				
	p	4	2	171.25	76.09	13	5.85
Sterilant	A	14	30				
	B	14	10				
	C	14	13				
	D	14	20	112.07	16.91	3	5.64
Batch	I	14	4				
	II	14	42				
	III	14	17				
	IV	14	10	154.93	59.77	3	19.92
Residuals +		-	-	-	143.07	36	3.97
Total		56	73	95.16	295.84	55	-

$T = 73$
$N = 56$
$T^2/N = 95.16$
$\Sigma x^2 = 391$

The residual variance includes that due to variations between plates and between specimens.

PHASE II

TABLE VII

Analysis of Variance in Number of Colonies on Bacteriostasis Controls

Factor	Level	n_*	T_*	$\sum T_*^2/n_*$	S_*	df	MS
Subject	a	16	2,903				
	e	16	1,330				
	f	16	2,450				
	g	16	2,425				
	h	16	1,921				
	i	16	912				
	j	16	1,327				
	k	16	1,660				
	l	16	2,073				
	m	16	1,508				
	n	16	1,687				
	o	16	1,561				
	o'	16	1,578				
	p	16	1,448	2,972,426	230,475	13	17,729
Plate	a	112	12,683				
	b	112	12,100	2,743,469	1,518	1	1,518
Dilution	0	112	13,081				
	1	112	11,702	2,750,441	8,490	1	8,490
Sterilant	A	56	6,489				
	B	56	7,710				
	C	56	5,437				
	D	56	5,147	2,814,354	72,403	3	24,134
Batch	I	56	7,474				
	II	56	7,984				
	III	56	7,857				
	IV	56	1,468	3,276,650	534,699	3	178,233
Residuals		-	-	—	1,428,929	202	7,074
Total		224	24,783	2,741,951	2,276,514	223	—

N_2 = 224
T^2_*/N = 2,741,951
$\sum x^2$ = 5,018,465
T = 24,783

PHASE II

TABLE XIII

Analysis of Variance in Change in Dimension

Factor	Level	n_*	T_*	$\sum T_*^2/n_*$	S_*	df	MS
Subjects	e	16	0.1613				
	i	16	3.8145				
	j	16	3.2318	1.5638	0.4815	2	0.2408
Replicates	1	24	3.5920				
	2	24	3.6156	1.0823	0.0	1	0.0
Sterilants	A	12	1.8235				
	B	12	1.8148				
	C	12	1.8137				
	D	12	1.7556	1.0825	0.0002	3	0.0001
Treatment	Before	24	3.5888				
	After	24	3.6188	1.0823	0.0	1	0.0
Residuals		-	-	-	0.0004	40	0.00001
Total		48	7.2076	1.0823	0.4821	47	-

$T = 7.2076$
$N = 48$
$T^2/N = 1.0823$
$\sum x^2 = 1.5644$

PHASE II

TABLE XVI

Analysis of Variance in Relative Change in Contact Resistance of Subjects k and k'

Factor	Level	n_*	T_*	$\sum T_*^2/n_*$	S_*	df	MS
Subject	k k'	16 16	651.4 -287.6	31,689.73	27,553.73	1	27,553.73
Measurement	a b	16 16	182.5 181.3	4,136.00	0.05	1	0.05
Replicate	1 2	16 16	- 11.9 375.7	8,830.76	4,694.81	1	4,694.81
Sterilant	A B C D	8 8 8 8	53.9 -138.4 178.7 269.6	15,834.70	11,698.75	3	3,899.58
Residuals		-	-	-	36,473.53	25	1,458.94
Total		32	363.8	4,135.95	80,420.87	31	-

$T = 363.8$

$N = 32$

$T^2/N = 4,135.95$

$\sum x^2 = 84,556.82$

PHASE II

TABLE XXIV

Analysis of Variance in Weight of Residue Extracted from Silicone Grease

Factor	Level	n_*	T_*	$\sum T^2/n_*$	S_*	df	MS
Replicate	1	4	949.2				
	2	4	748.8	365,420.52	5,020.02	1	5,020.02
Sterilant	A	2	845.1				
	B	2	467.4				
	C	2	303.8				
	D	2	81.7	515,813.05	155,412.55	3	51,804.18
Residuals		-	-	-	17,418.79	3	5,806.26
Totals		8	1,698.0	360,400.50	177,851.36	7	-

$N = 8$

$T = 1,698.0$

$T^2/N = 360,400.50$

$\sum x^2 = 538,251.86$

PHASE V

TABLE I

1/9 Fractional Factorial Design of 5 Variables
Positive Tubes §

		A_0									A_1									A_2								
		B_0			B_1			B_2			B_0			B_1			B_2			B_0			B_1			B_2		
		C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2
D_0	E_0	0*					3*		1*																			
	E_1											14		20					0									
	E_2																					1^+		6^+		5^+		
D_1	E_0																			14*					0*		20*	
	E_1		22		21					1																		
	E_2												0^+		6^+		7^+											
D_2	E_0										5**					1*		9*										
	E_1																				24		24					1
	E_2			0^+		0^+		4^+																				

* Neither the growth control nor the bacteriostasis control grew.

\+ The growth controls grew at 10^8 and 10^7 inoculums, but not at 10^6 inoculum, while only a few of the bacteriostasis controls grew.

** The growth control grew at the 10^8 inoculum while the bacteriostasis control did not grow.

§ After 14 days incubation at the appropriate temperature.

PHASE V

TABLE IV

Analysis of Variance of Cl. sporogenes Sterilization

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS
Column	A B C	72 72 72	60 65 2	108.7	34.0	2	17.0
Rows	α β γ	72 72 72	34 44 49	76.3	1.6	2	0.8
Residual	-	-	-	-	75.2	211	0.7
Total	-	216	127	74.7	192.8	215	-

$N = 216$ $\quad T^2/N = 74.7$

$T = 127$ $\quad \Sigma x^2 = 267.5$

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